DIFFERENT ANALYTICAL METHODS FOR THE DETERMINATION OF NIFEDIPINE IN THE TABLET FORMULATION

Dissertation

Submitted to

THE TAMILNADU Dr. M. G. R. MEDICAL UNIVERSITY,

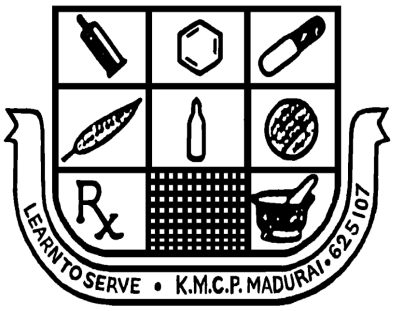
CHENNAI

In partial fulfillment for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL ANALYSIS



DEPARTMENT OF PHARMACEUTICAL ANALYSIS

K. M. COLLEGE OF PHARMACY

MELUR ROAD, UTHANGUDI

MADURAI - 625107

APRIL - 2014

CERTIFICATE

This is to certify that the dissertation entitled **"DIFFERENT ANALYTICAL METHODS FOR THE DETERMINATION OF NIFEDIPINE IN THE TABLET FORMULATION"** by **SASI RUBA. C (Reg. No. 261230054)** in partial fulfillment of the degree of **Master of Pharmacy** in **Pharmaceutical Analysis** under The Tamil Nadu Dr. M. G. R. Medical University, Chennai, done at **K. M. COLLEGE OF PHARMACY**, MADURAI - 625107, is a bonafide work carried out by her under my guidance and supervision during the academic year APRIL 2013-2014. The dissertation partially or fully has not been submitted for any other degree or diploma of this university or other universities.

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I might have forgotten to name a few people, behind this work, but still really thank to all concerned individuals for their support to complete this work successfully in time.

THANKS TO ALL WITH MEMORY

C. SASI RUBA

CONTENTS

ABBREVIATIONS

%	-	Percentage
QS	-	Quinine sulphate
λ	-	Lambda
μg	-	Microgram
w/w	-	Weight by weight
v/v	-	Volume by volume
$\mu g / ml$	-	Microgram per milliliter
Conc	-	Concentration
mg	-	Milligram
ml	-	Milliliter
nm	-	Nanometer
UV- Vis	-	Ultra violet-Visible
Em	-	Emission
Ex	-	Excitation
ng	-	Nanogram
RP-HPLC	-	Reverse phase High Performance Liquid Chromatography
Std	-	Standard
SD	-	Standard Deviation
Wt	-	Weight
LOD	-	Limit of Detection
LOQ	-	Limit of Quantitation
F.I	-	Fluorescence Intensity

NF	-	Nifedipine
HPTLC	-	High-Performance Thin Layer Chromatography
HPLC	-	High-Performance Liquid Chromatography
LC	-	Liquid Chromatography
GC	-	Gas Chromatography
LC-MS	-	Liquid Chromatography-Mass spectrometry
RSD	-	Relative Standard Deviation
GC-MS	-	Gas Chromatography-Mass Spectrometry
ICH	-	International Conference on Harmonization
AR	-	Analytical reagent
e.g.	-	Example gratitis
FDA	-	Food and Drug Administration
gm	-	Gram
S. No	-	Serial number
^{0}C	-	Degree centigrade
CE-MS	-	Capillary Electrophoresis-Mass Spectrometry
PMT	-	Photo multiplier tube
WHO	-	World Health Organization

INTRODUCTION

PHARMACEUTICAL ANALYSIS

Pharmaceutical analysis is an interdisciplinary subject and derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics.

Analytical determination is based on the measurement of some physical, chemical or structural properties which are related directly or indirectly to the amount of desired constituent present in the sample.

DRUG

Drug is referred to as medicine or medication, can be loosely defined as any chemical substance intended for use in the medical, diagnosis, cure, treatment, or prevention of disease. Drugs are the chemicals which are originated from either organic or inorganic. Some property of medicinal agent is used to measure them qualitatively or quantitatively.

The Pharmaceutical analysis is intended as a collection of all methods and procedures on all possible pharmaceutical compounds. Both classical methods as well as instrumental methods of analysis are required in quality control work. In modern pharmaceutical analysis separation techniques such as HPLC, HPTLC and GC are employed nowadays for the analysis of drugs in biological fluids, raw materials and dosage forms. The analysis of drugs by functional group reactions may be required to provide sensitivity and selectivity purpose. The electro analytical methods of analysis are advantageous to analyse certain group of pharmaceuticals but nowadays the use of electrochemical methods are employed for detecting analytes in HPLC eluates.

The electronic absorption spectroscopy in near UV and Visible wavelength regions is a familiar approach in the analysis of active pharmaceutical ingredients and the formulations containing them. However it lacks the information of relationship of structure of analyte and the environment of the molecule to the observed spectrum. Fluorescence spectroscopy is well known and widely used technique in chemical analysis. This technique is limited in the quantification of pharmaceuticals possessing fluorescent character. However in recent days non-fluorescent molecules have been quantified employing indirect procedures such as chemical derivitization,

tagging with a fluorescent molecule or by quenching ability of certain chemicals. Radio chemical methods are other catogories of pharmaceutical analysis employed for radio pharmaceuticals. Radio immuno assay promises to be a powerful analytical technique for determining trace level of drugs in complex biological matrices. The classical methods of analysis such as titrimetric methods and gravimetric methods have been restricted in the determination of bulk inorganic pharmaceuticals and excipients.

Quality assurance plays a important role in determining the safety and efficacy of medicine. Highly specific and sensitive analytical techniques are important in the design development, standardisation and quality control of medicinal products. They are also important in pharmacokinetics and drug metabolism studies. Modern instrumental methods are extremely sensitive providing precise and detailed information from small samples of material. All drug substances, their formulated dosage forms are subjected to strict quality assurance procedures. Pharmacokinetic studies often requires measurements in the microgram, nanogram or picogram/ml range. Powerful coupled separative-detector system such as GC-MS, LC-MS, CE-MS are used. The above systems are extremely versatile and expensive much valuable work can be still accomplished with the help of UV-spectrophotometry, spectrofluorimetry and polarography.

ANALYTICAL CHEMISTRY:

It can be defined as the science and art of determining the composition of materials ,in terms of the elements or compounds contained either qualitatively or quantitatively.[42] It is concerned with the identification of a substance, the elucidation of its structure and quantitative analysis of its composition. [43] It is divided into two parts,

A . Qualitative analysis

B . Quantitative analysis

Qualitative Analysis:

Qualitative analysis refers to the identity of the drug or identity of the elements radicals or functional groups present in the compounds.

Quantitative Analysis:

Quantitative analysis involves determination of percentage purity of raw drugs or quantity of drug present in the given dose of formulation. To achieve the above aspects different analytical methods are needed.

TYPES OF ANALYSIS:

MODERN CLASSIFICATION:

1. **Classical method of analysis**
   - Acid - Base titration
   - Redox titration
   - Complexometric titration
   - Non aqueous titration
   - Diazotisation titration
   - Gravimetry

2. **Instrumental method of analysis**
   - Electro analytical techniques
   - Spectroscopic techniques
3. **Separation technique**
   - Chromatography - hyphenated techniques

4. **Biological / Microbiological method of analysis**

   - Microbiological assay
   - Bio assay
5. **Radiochemical methods**

1. **Classical method of analysis:**

It can be subdivided into two types titrimetry and gravimetry. Titrimetry method of analysis involves use of standard reagent. The analyte in solution is made to react with definite volume of the standard reagent of known concentration.

2. **Instrumental method of analysis:**

(a) Electro analytical techniques involves,

- Potentiometry
- Amperometry
- Conductometry
- Coulometry

SPECTROSCOPY:

Spectroscopy is the measurement and interpretation of electromagnetic radiation absorbed, scattered or emitted by atoms, molecules or other chemical species.The absorption or emission is associated with changes in energy states of the interacting chemical species and since each species has characteristic energy states, spectroscopy can be used to identify the interacting species.

The principle types of spectroscopic techniques are,

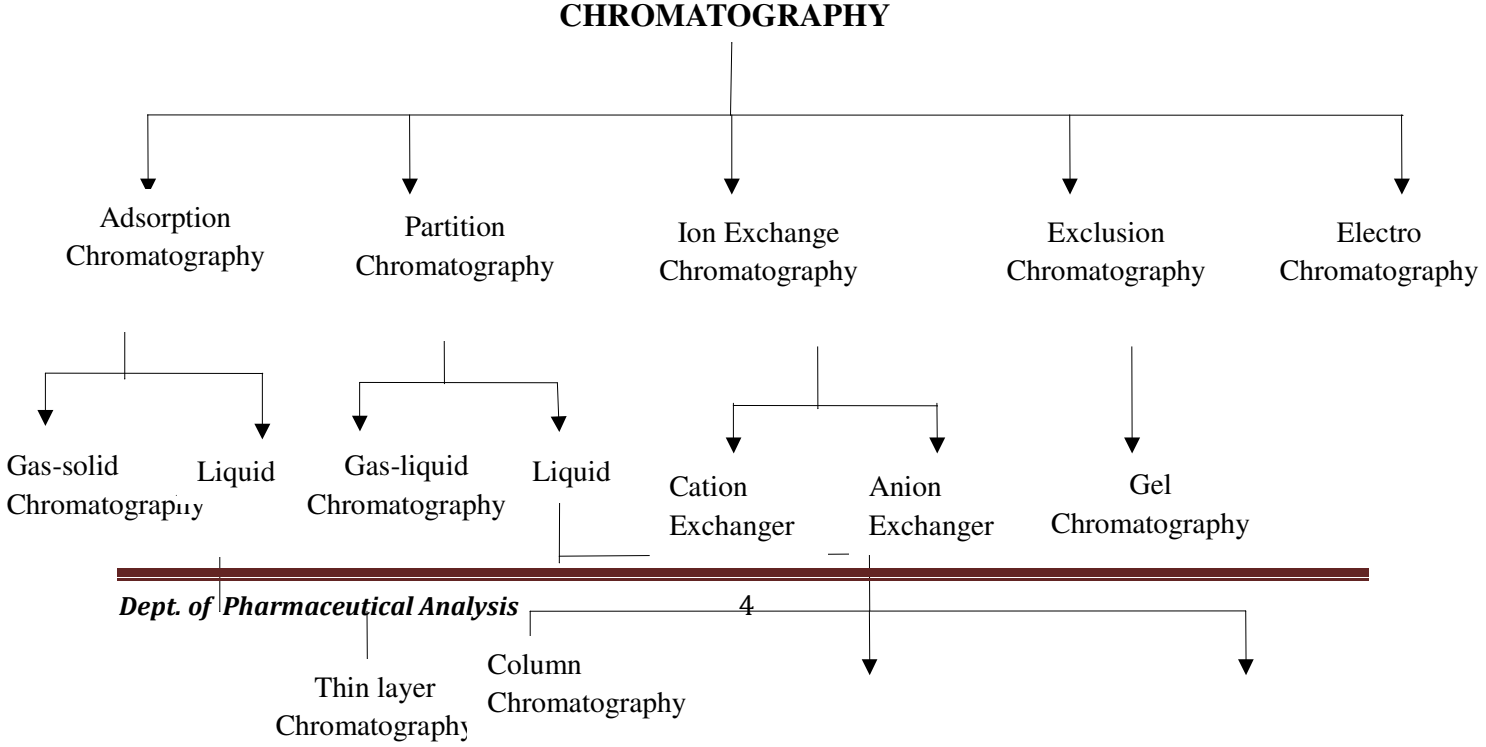
- Ultraviolet and visible spectroscopy
- Fluorescence and phosphorescence spectroscopy
- Atomic spectroscopy (emission and absorption)
- Infrared spectroscopy
- X-ray spectroscopy
- Nuclear magnetic resonance spectroscopy
- Electron spin resonance spectroscopy

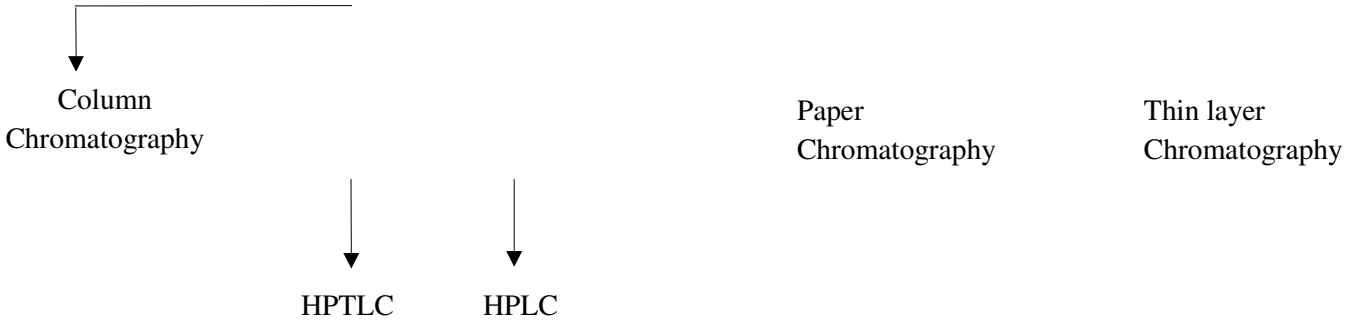
3. SEPARATION TECHNIQUES:

Chromatography:

Chromatography is a group of techniques for the separation of the compounds of mixtures by their continuous distribution between two phases one of which is moving past the other .[1b]

FLOW CHART TYPES OF CHROMATOGRAPHY [6]





Hyphenated - Techniques :

- Gas Chromatography - Mass Spectroscopy (GC - MS)
- Liquid Chromatography - Mass Spectrometry (LC - MS)
- Liquid Chromatography - Nuclear Magnetic Resonance
- Liquid Chromatography - Fourier Transform Infrared

ULTRAVIOLET AND VISIBLE ABSORPTION SPECTROPHOTOMETRY

Spectroscopy is the study of interaction of matter with electromagnetic radiation. A beam of photons constitute electromagnetic radiations (EMR) in which photons are distributed in energy bundles. Based on wavelength, frequency and energy of radiation, the electromagnetic spectrum is divided into following rays:

Cosmic rays
Gamma rays
X-rays
Ultra-violet rays
Visible rays
Infrared rays
Microwave rays
Radio waves

The UV region in electromagnetic spectrum is from 200 nm to 400 nm and the visible region in electromagnetic spectrum is from 400 nm to 800 nm. The two laws which govern the UV -Visible spectroscopy are Beer's law and Lambert's law.

Beer's law states that the rate of decrease of intensity of radiation with concentration of absorbing species is proportional to the intensity of incident radiation.

The absorbance A, of a solution is defined as the logarithm to base $_{10}$ of the reciprocal of the transmittance T, for monochromatic light, and is expressed by the equation:

$$\mathbf{A = \log_{10}(I/T) = \log_{10}(I_0/I)}$$

where I = The intensity of transmitted monochromatic light,

I_0 = The intensity of incident monochromatic light and $T = I/I_0$

In the absence of other physio-chemical factors, the measured absorbance (A) is proportional to the path length (d) through which the light passes and to the concentration (c) of the substance in accordance with the expression

$$\mathbf{A = cd}$$

where ε is the molar absorptivity when d is expressed in cm and c in moles per litre.

The expression A(1%, 1 cm) representing the specific absorbance of a dissolved substance refers to the absorbance of a 1.0% w/v solution in a 1 cm cell and measured at a defined wavelengh so that

$$\mathbf{A(1\%, 1\ cm) = 10\varepsilon/M}$$

where M is the molecular weight of the substance being examined.

The specific absorbance is therefore the notional absorbance of a 1-cm layer of a 1% w/v solution of the absorbing solute, its value at a particular wavelength in a given solvent being a property of the solute. The absorbance of the solution is usually measured in a cuvette with 1 cm path length.

In measuring the absorbance of a solution at a given wavelength, the absorbance of the reference cell and its contents should not exceed 0.4 and is preferably less than 0.2 when measured with reference to air at the same wavelength.

The absorption spectrum is plotted with absorbance or function of absorbance as ordinate against wavelength of function of wavelength as abscissa. [8]

Apparatus :

Spectrophotometers suitable for measuring in the ultraviolet and visible range of the spectrum consist of an optical system capable of producing monochromatic light in the range 200 nm to 800 nm and a device suitable for determining the absorbance.

Instrument Components :

- Light source
- Monochromator
- Sample / Reference cells
- Detector
- Signal processors and readout system

Light Source :

Tungsten Filament lamp - for production of visible radiation.

Hydrogen - Deuterium discharge lamp - for production of UV radiation.

Monochromator :

The various wavelength from light source are separated with a prism or grating and then selected by slits such that the rotation of the prism causes a series of continuously increasing wavelengths to pass through the slits for recording purposes. The selected beam is monochromatic which is then divided into two beams of equal intensity.

Sample / Reference cells :

Sample / Reference can be contained in cells made of a material which is transparent throughout the region under study. Glass cannot be used since it absorbs strongly in the ultra violet region because glass are made up of alkali earth metals. Quartz are used in UV region. Glass can be satisfactorily used in visible region.

Detectors :

Photomultiplier tubes and photo diodes are the two important type of photo detectors. For the determination of substances by spectrophotometric techniques, precise determinations of the light intensities are necessary. Photoelectric detectors are most frequently used for this purpose. They must be employed in such a way that they give response linearly proportional to the light input and they must suffer from drift . [1b]

Signal processors and readoutsystem :

A signal processors is an ordinarily an electronic device that amplifies the electric signal from the detector. In addition it may alter the signal from ac to dc, change the phase of signal and filter it to remove unwanted components. The signal processors may also be called on to perform such mathematical operation on the signal differentiation, integration or convert to logarithm.

SPECTROFLUORIMETRY

Introduction:

When the molecules are exposed to the electromagnetic radiation they exhibit fluorescence. Fluorescence is a process of re-emission of radiation energy absorbed in the form of visible or UV light. In this process, the light emitted is always of longer wavelength than that absorbed. In fluorescence, absorption and emission of light takes place in short time (10^{-12} to 10^{-9} seconds). The quantitative determination of fluorescence is the basis of fluorimetric analysis. [(2)]

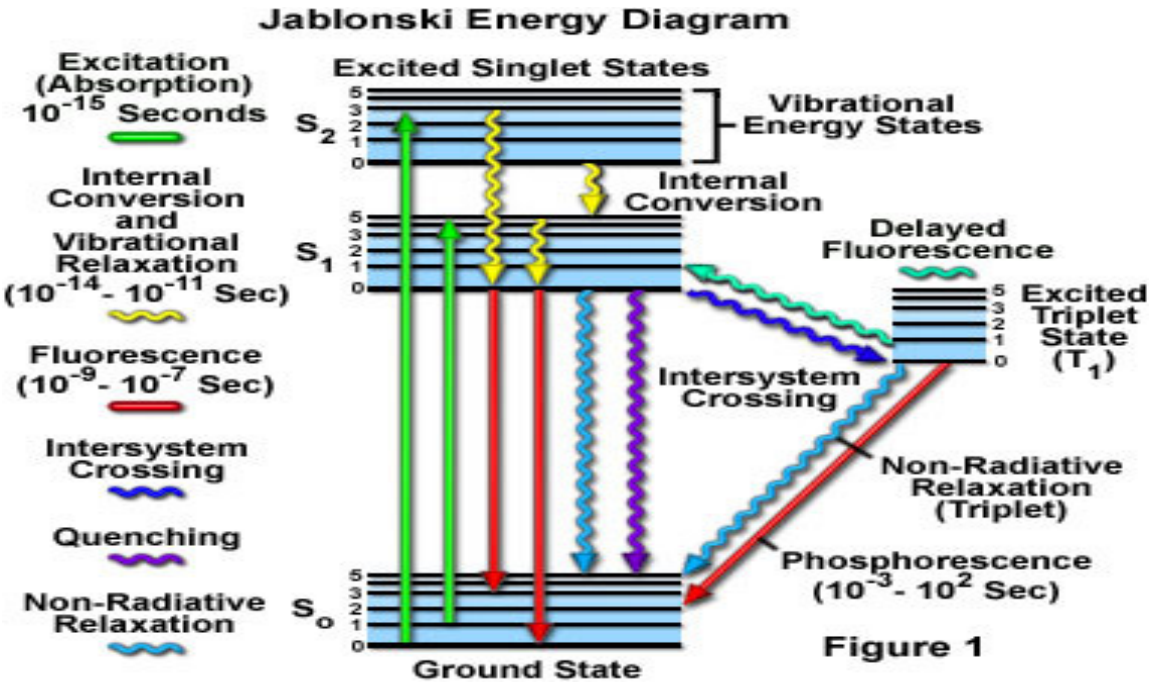
Figure No. 1 Spectrofluorimeter

Principle of fluorescence :

A molecule in a ground state is considered to have three energy levels viz. rotational, vibrational and electronic. When an electromagnetic radiation falls on the molecule it brings changes into its changes into its energy levels and they will go to excited state. The excited molecular species are extremely short-lived and deactivation occurs due to:

- Internal collisions
- Cleavage of chemical bonds, initiating photochemical reactions
- Re-emission as light (luminescence)

Figure No. 2



Molecules on excitation normally possess higher vibrational energy than they had in the ground state. This extra vibrational energy is lost by collision, after which the molecule returns to the ground state with the emission of light as fluorescence. Fluorescence is a rapid process occurring within 10^{-6} to 10^{-9} seconds of the excitation. If there is any delay in the emission of light is called as phosphorescence. The delay period may range from fraction of a second to few days. Both these processes of re-emission are generally designated as luminescence. [3]

Factors Affecting Fluorescence :

$$F = 2.3\ I_o\ abc\ \phi_j$$

For a fixed set of instrumental (I_o and b) and sample (a and ϕ_j) parameter, the fluorescence is proportional to concentration.

$$F = kc \text{ where } k = 2.3\ I_o ab\ \phi_j$$

There are number of factors which or directly or indirectly affect the fluorescence and decrease its intensity and sensitivity which is termed as "quenching".

1. Concentration :

Fluorescence is best in dilute solution. In concentrated solution the intensity of fluorescence is reduced and is not quantitative. This is called as concentration quenching.

2. Quantum yield of fluorescence (ϕ_j):

ϕ_j = no of photons emitted / no of photons absorbed

Highly fluorescent substances have ϕ_j values near to unity, which shows that most of the absorbed energy is re-emitted as fluorescence. Non fluorescent substance have ϕ_j =0.

3. Intensity of Incident light :

An increase in the intensity of light incident on the sample produces a proportional increase in the fluorescent intensity. The intensity of incident light and sensitivity of fluorescence measurement are increased by increasing the width of the excitation slit.

4. Oxygen :

The presence of oxygen may interfere in two ways, by direct oxidation of the fluorescent to non-fluorescent products, or by quenching of fluorescence.

5. pH :

Alteration of pH brings in the ionized and non-ionized form of fluorogenic material.

6. Temperature and viscosity :

An increase in temperature or decrease in viscosity is likely to decrease fluorescence by deactivation of the excited molecules by collision. Low temperature and appropriate dilute solution are necessary for quantitative analysis.

7. Quenchers :

Collisional quenchers :

Collisional quenchers reduce fluorescence by dissipating absorbed energy as heat due to collisions with the quenching species.

e.g. Quinine is highly fluorescent in 0.05 M Sulphuric Acid but non-fluorescent in 0.1 M Hydrochloric Acid or in presence of halide salt.

Static quenchers :

Static quenchers form a chemical complex with the fluorescent substance and alter its fluorescence characteristics.

e.g. Caffeine reduces the fluorescence of riboflavin by static quenching.

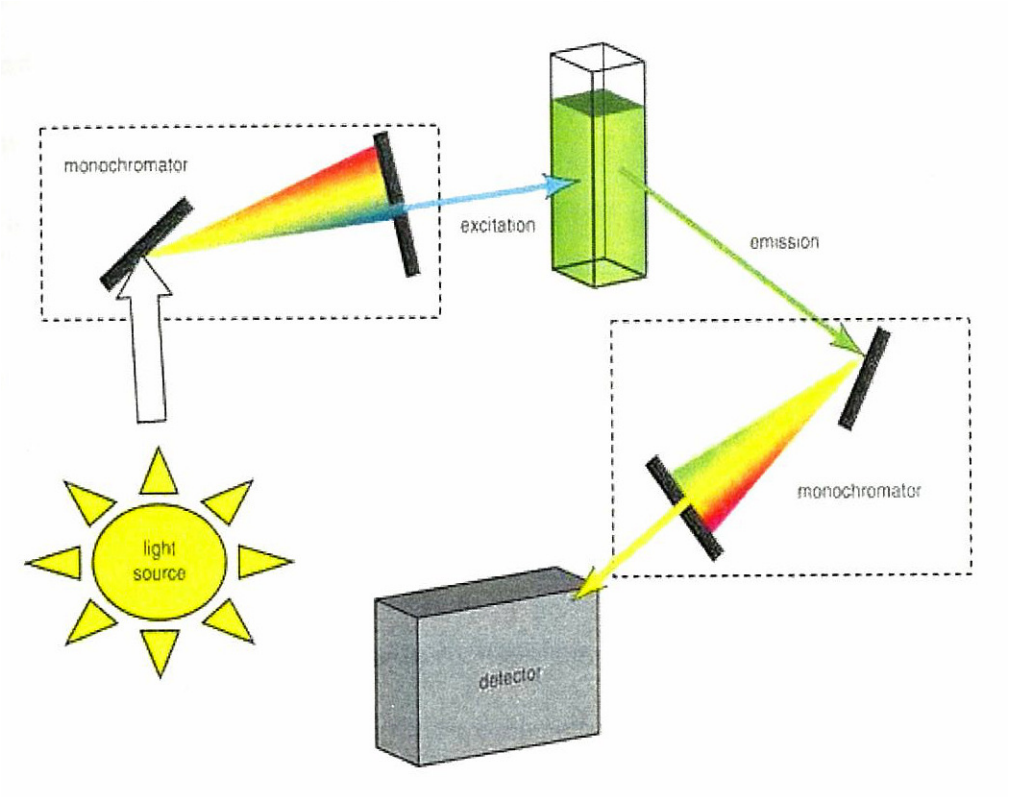
Instrumentation :

Two primary kind of instruments are used to measure the fluorescence

- Filter fluorimeter
- Spectrofluorometer

Instruments that measure the intensity of fluorescence are called fluorimeters. Those that measure the fluorescence intensity at variable wavelength of excitation and emission and are able to produce fluorescence spectra are called as Spectrofluorimeters. [1a]

Figure No. 3 Components of a spectrofluorimeter



1. Source of light :

- Mercury vapour lamp (or)
- Xenon-arc lamp
  Providing light output from 190 - 1200 nm
  Versatile and powerful

2. Filter and monochromator :

In spectrofluorometer, excitation and emission monochromators are used

Excitation Monochromator :

Provides a suitable radiation for excitation of the molecules

Emission Monochromator :

Isolate only the radiation emitted by the fluorescent molecule

3. Cuvettes/cells :

Four sides polished cuvettes are used. Made up of borosilicate or quartz glass.

4. Detector :

Photo multiplier tube (PMT) is used as detector.

Advantage :

- Standard device
- Large signal
- Fast rise time possible

Disadvantage :

- High voltage required
- Sensitive to magnetic field

The light source produces light photons over a broad energy spectrum, typically ranging from 200 to 900 nm. When photons falls on the excitation monochromator, which selectively transmits light in a narrow range. The transmitted light passes through adjustable slit that control magnitude and resolution by further limiting the range of transmitted light. The filtered light passes in to the sample cell causing fluorescent emission by fluorophores with in the sample. Emitted light enters the emission monochromator, which is positioned at 90^0 angle from the excitation light path to eliminate background signal and minise noise due to stray light. Again the

emitted light is transmitted through adjustible slits, finally entering the PMT. The signal is amplified and create a voltage that is proportional to the measured emitted intensity. [7]

Advantages of fluorimetry :

- Hundred to thousands times more sensitive than absorbance techniques
- Possible to analyse sample in the nanogram to picogram level

VALIDATION

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. [37]

Validation is not a method development tool and it does not make a method good or efficient. [38]

Validation is a team effort. It will involve people from various functions in the plant. [39]

Validation is defined as follows by different agencies,

Food and Drug administration (FDA) [40] :

Establishing documentation evidence, which provides a high degree of assurance that specific process, will consistently produce a product meeting its predetermined specification and quality attributes.

World Health Organization (WHO) :

Action of providing that any procedure, process, equipment, material, activity, or system actually leads to the expected results.

European Committee [41] :

Action of providing in accordance with the principles of good manufacturing practice that any procedure, process, equipment, material, activity, or system actually leads to the expected results. In brief validation is a key process for effective quality assurance.

Reasons for Validation :

There are two important reasons for validating assays in the pharmaceutical industry. The first, and by for the most important, is that assay validation is an integral part of the quality-control system. The second is that current good manufacturing practice regulation requires assay validation.

Method Validation parameters :

1. Accuracy

2. Precision
3. Repeatability
4. Intermediate precision
5. Reproducibility
6. Range
7. Specificity
8. Linearity
9. Limit of detection (LOD)
10. Limit of quantitation (LOQ)
11. Ruggedness
12. Robustness

1. Accuracy :

The closeness of agreement between the value, which are accepted either as a conventional true value or accepted reference value and the value found. The accuracy of the method is tested by recovery studies at three different levels by adding a known amount of pure drug to the pre-analysed formulation of definite concentration.

2. Precision :

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions. ICH divides precision into three types.

1. Repeatability
2. Intermediate precision
3. Reproducibilty

3. Repeatability :

Repeatability is the precision of method under the same operating conditions over a short period of time. One aspect of this is instrumental precision. A second aspect is sometimes termed intra-assay precision and involves multiple measurements of the same sample by the same analyst under the conditions.

4. Intermediate precision :

Intermediate precision is the agreement of complete measurements when the same method is applied many times within the same laboratory. This can include full analysis samples and standards.

5. Reproducibility :

Reproducibility examines the precision between laboratories and is often determined in collaborative studies or method transfer experiments.

Precision often is expressed by the standard deviation or relative standard deviation of the data set.

6. Range :

The range of a method can be define as upper and lower concentration for which the analytical method has adequate accuracy, precision and linearity. The range of concentration examined will depended on the type of method and its use.

7. Specificity :

Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. Assuring specificity is the first step in developing and validating a good method. If specificity is not assured, method accuracy, precision and linearity all are seriously compromised. Method specificity should be reassessed continually during validation and subsequent use of method.

8. Linearity :

The linearity of an analytical procedure is its ability to obtain test result, which are directly proportional to concentration of analyte in the sample. Linearity can be assessed by performing single measurements at several analyte concentration. A linearity correlation coefficient above 0.999 is acceptable for most methods, especially for major components in assay method.

9. Limit of detection (LOD) :

The limit of detection an individual analytical procedure is the lowest amount of analyte in the sample which can detected but not necessarily quantitated as an exact value. The limit of detection (LOD) may be expressed as;

$$LOD = \sigma\ 3.3 / S$$

Where

σ = the standard deviation of the response

S = the slope of the calibration curve of the analyte

10. Limit of quantitation (LOQ) :

The limit of quantitation of an analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy.

Limit of quantitation (LOQ) can be expressed as;

$$LOQ = \sigma 10 / S$$

Where,

σ = standard deviation of the response

S = slope of the calibration curve of the analyte

11. Ruggedness :

Method ruggedness is defined as the reproducibility of result when the method is performed under actual use conditions. This includes different analysts, laboratories and instruments. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method.

12. Robustness :

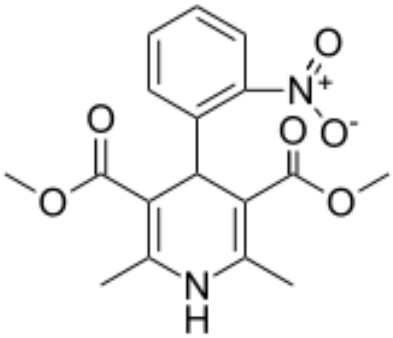
The concept of robustness of an analytical procedure has been defined by the ICH as "a method of its capacity to remain to unaffected by small, but deliberate variation in method parameters". The robustness of the method was tested by changing wavelength range, scanning speed and solvents composition.

2. DRUG PROFILE

NIFEDIPINE

2.1 NOMENCLATURE

Structure :



Chemical Formula :

3,5-dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate

CAS Registry number :

21829-25-4

Molecular Formula :

$C_{17}H_{18}N_2O_6$

Molecular Weight :

346.335 g/mol

Category :

Antianginal and Antihypertensive

ATC code :

C08CA05

2.2 PHYSICAL PROPERTIES :

Nifedipine is a yellowish crystalline powder. It is stored at room temperature. It melt in the range of 171 - 175 ºC. It is insoluble in water, freely soluble in ethanol, methanol, and in acetone.

2.3 DOSAGE FORMS :

Formulations currently available are Nifedipine in ordinary capsules (5 and 10 milligrams), soft gel capsules (5 mg and 10 milligrams), coated tablets (10, 30, 40 and 60 milligrams) and extended release tablets (5, 10, and 20 milligrams).

2.4 PHARMACOLOGY :

Indication :

Nifedipine is indicated in angina (chest pain), high blood pressure and abnormal heart rhythms. Nifedipine works by relaxing blood vessels.

Mechanism of Action :

Nifedipine is a calcium channel blocker. It has two mechanism of action. They are,

- Relaxation and Prevention of Coronary Artery Spasm
- Reduction of oxygen utilization

Relaxation and Prevention of Coronary Artery Spasm :

Nifedipine dilates the main coronary arteries and coronary arterioles, both in normal and ischemic regions, and is a potent inhibitor of coronary artery spasm, whether spontaneous or ergonovine-induced. This property increases myocardial oxygen delivery in patients with coronary artery spasm, and is responsible for the effectiveness of nifedipine in vasospastic angina.

Reduction of Oxygen Utilization :

Nifedipine regularly reduces arterial pressure at rest and at a given level of exercise by dilating peripheral arterioles and reducing the total peripheral resistance (afterload) against which the heart works. This unloading of the heart reduces myocardial energy consumption and oxygen requirements and probably accounts for the effectiveness of nifedipine in chronic stable angina.

Contraindication :

It is contraindicated in patients with very low blood pressure or shock due to heart problems and hypersensitivity.

Special Precaution :

It should not be given to patients over the age of 65 and above and pregnancy cases.

Adverse Effects :

The adverse drug reactions are headache, feeling of lightheadedness, sleepy, blurred vision, and lowered concentration, hot flushes, headache, sudden change of position may cause dizziness, nausea and vomiting, constipation or diarrhea, swelling of extremities, and gum problems.

Drug Interactions :

Alcohol	-	It will increase drowsiness
Grapefruit	-	This will increase the incidence of side effects
Cyclosporin	-	It increases risk for gingivitis
Beta-adrenergic blockers	-	It increases risk for congestive heart failure, severe lowering of blood pressure and further aggravation of chest pain might arise

2.5 PHARMACOKINETICS :

Absorption :

- Nifedipine is rapidly and fully absorbed in oral administration.
- Nifedipine is detectable in serum 10 minutes after oral administration. Peak blood level occurs approximately in 30 minutes.

Distribution :

92-98 % of Nifedipine is bound to serum protein.

Metabolism :

The drug is bio transformed in liver. The principal metabolite is 2,6-dimethyl-4-(2-nitrophenyl)-5-methoxycarbonyl-pyridine-3-carboxylic acid .

Excretion :

The elimination half life is approximately 2 hours.

LITERATURE REVIEW

SPECTROSCOPIC METHODS :

R K Sheladia *et al.,* [2011] have reported spectrophotometric determination of nifidipine in bulk and tablet dosage form. The method was based on reduction of the nitro group of nifedipine to amino group by treatment with zinc dust and hydrochloric acid. The reduced molecule was diazotized with sodium nitrite and coupled with Bratton-Marshall reagent N-(1-naphthyl) ethylenediamine dihydrochloride. The absorbance was measured for the magenta coloured derivative at 430 nm. The method was validated in terms of precision, specificity and lineatity range. [9]

R.Revathi *et al.,* [2010] developed a spectroscopic determination of nifedipine using 40 % sodium salicylate solution as hydrotropic solubilizing agent. The drug showed λ-max at 350 nm and beer's law was obeyed in the concentration range of 20 – 100 µg/ml. The result of analysis for tablet dosage form obtained by the proposed method was compared with pharmacopoeial method. The proposed method was statistically validated as per ICH guidelines. The percentage content and percentage recoveries estimated for Nifedipine marketed tablet formulations were close to 100 with low %RSD. [10]

Sathis Kumar Dinakaran *et al.,* [2013] have studied and reported a simple, economic, accurate absorption ratio method for the simultaneous estimation of atorvastatin calcium and nifedipine HCl in bulk and tablet dosage form. Methanol was used as a diluent. The absorption maxima were observed at 237 nm and 297 nm which were selected based on overlap spectra of atorvastatin calcium and nifedipine HCl.. The proposed method was validated. [11]

Sheikha M.Al-Ghannam *et al.,* developed a spectrofluorometric determination of nicardipine,nifedipine and isradipine in pharmaceutical preparations and in biological fluids. The method was based on the reduction of nicardipine, nifedipine and isradipine with Zn/HCl and measuring the fluorescence intensity of reduction product ($\lambda_{em}/\lambda_{ex}$) at 460/364, 450/393 and 446/360 nm, respectively. The factors affecting the development of the fluorophore and its stability were studied and optimized. The effect of some surfactants such as β-cyclodextrin (βCD), carboxymethylcelullose (CMC), sodium dodecyl sulphate (SDS) and triton ×-100, on the

fluorescence intensity was also studied. The fluorescence intensity-concentration plots of nicardipine, nifedipine and isradipine were rectilinear over the ranges 0.4–6.0, 0.2–4.0 and 0.1–9.0 μg ml^{-1} with detection limits of 0.0028, 0.017 and 0.016 μg ml^{-1}, respectively. The proposed method was successfully applied to commercial tablets containing the compounds and the percentage recovery agreed well with those obtained using the official methods. The method was further extended to the in vitro determination of the compounds in spiked human plasma and urine samples. [12]

B.Hemmateenejad *et al.,* [2008] have reported a simple, accurate, sensitive and economical colorimetric procedure for the estimation of amlodipine besylate and nifedipine, both in pure and dosage forms. The method was based on the reduction of iron(III) by the studied drugs and subsequent interaction of iron(II) with ferricyanide to form prussian blue. The reaction developed through a slow kinetics and completes in about 10 min. Both initial slope and fixed time methods were used to derive calibration graphs. The resulted calibration equations were linear in the concentration ranges of 1.0-20.0 and 3.0-19.0 μg ml-1 for AML and NIF, and the detection limits were 0.10 and0.19 μg ml-1, respectively. Seven replicate analyses of solutions containing three different levels of each drug resulted in very low relative error of prediction (less than 1.6%) and relative standard deviation (less than 4%) confirming accuracy and precision of the proposed method. [13]

Sable Kunal *et al.,* [2012] have described UV-Spectrophotometric method for the simultaneous determination of nifedipine and atenolol in combined dosage form as well as in laboratory mixture was studied under this paper. Nifedipine and Atenolol are used in combined dosage form for Cardiovascular System Diseases. The developed method was validated as per ICH guidelines. The stability study of combined dosage form was carried out by using the IR spectrum of both the drug. [14]

Normaizira Hamidi *et al.,* [2008] have reported analysis of nifedipine content in transdermal drug delivery system using non-destructive visible spectrophotometric technique. The applicability of visible spectrophotometry technique as a tool to determine the drug content of polymeric film was investigated. Hydroxypropylmethycellulose (HPMC) was selected as the matrix polymer and nifedipine as the model drug. Blank and nifedipine-loaded HPMC films were prepared using the solvent evaporation method. The absorbance spectra of these films under the visible wavelengths between 400 and 800 nm were assessed and compared against the

drug content values obtained by means of the conventional destructive UV spectrophotometry technique. The results indicated that the absorbance values, attributed to nifedipine, at the wavelengths of 545, 585, 638 and 755nm were significantly correlated to the drug content values obtained using the chemical assay method (Pearson correlation value: $r \geq 0.990$ and $p < 0.01$). The visible spectrophotometry technique was potentially suitable for use to determine the nifedipine content of films. The samples were recoverable from test and analysis of the entire batch of samples was possible without the need of solvents and chemical reagents. [15]

Joaquim C.G. Esteves da Silvab *et al.,* [2010] have investigated fluorescence derivatization reaction and quantification of the antihypertensor nifedipine. A fluorescence derivatization reaction based on the reaction of Nifedipine with o-phthalaldehyde after a previous reduction of the nitro group to a primary amine and photooxidation of the dihydropyridine to pyridine was optimized. Chemometric data analysis was done by parallel factor analysis 2 (PARAFAC2) multivariate calibration model of a three way data structure obtained by the acquisition of complete excitation-emission matrices (EEMs) of a Nifedipine fluorescent derivate following a standard addition method. The results found in the quantification of Nifedipine in different pharmaceutical formulations were compared with the official US Pharmacopeia high performance liquid chromatography - ultraviolet method (USP HPLC-UV). For each of the pharmaceutical formulations, a fortified sample was also analysed. Good results were found with the PARAFAC2 spectrofluorimetric methodology. Generally, similar PARAFAC2 models were needed for the adequate decomposition of the unfortified and fortified samples of the same pharmaceutical formulation. [16]

Nafisur Rahman *et al.,* [2005] have described two simple , sensitive and economical sepctrophotometric methods were developed for the determination of nifedipine in pharmaceutical formulations. Method A was based on the reaction of the nitro group of the drug with potassium hydroxide in dimethyl sulphoxide (DMSO) medium to form a coloured product, which absorbed maximally at 430 nm. Method B used oxidation of the drug with ammonium molybate and subsequently reduced molybdenum blue was measured at 830 nm. Beer's law was obeyed in the concentration range of 5.0-50.0 and 2.5-45.0 μg ml^{-1} with methods A and B ,respectively. Both methods have been successfully applied for the assay of the drug in pharmaceutical formulations. No interference was observed from common pharmaceutical

adjuvants. The reliability and the performance of the proposed methods were established by point and interval hypothesis tests and through recovery studies.[17]

Olajire Aremu Adegoke *et al.,* [2012] have described a new sensitive colorimetric method for nifedipine following azo dye formation with 4-carboxyl-2,6-dinitrobenzenediazonium ion (CDNBD). Judging from the various generic brands currently available, this research was conceived as a means of developing an alternative cost-effective and readily adaptable method for the assay of nifedipine in tablets and for which official high performance liquid chromatographic technique may not be readily available. Nifedipine was reduced with Zn/Hcl reduction system and then the diazo coupling reaction was carried out with the CDNBD reagent to generate a new azo adduct with optimal wavelength at 470nm representing a bathochromic shift relative to nifedipine, reduced nifedipine and CDNBD reagent. Optimal temperature and time for coupling were selected as 50^0C and 15 minutes. A linear response was observed over2.9-14.5microgram per ml of nifedipine. The developed spectrophotometric method is of equivalent accuracy ($p>0.005$) with USP 2007 HPLC method.[18]

Sayed M.Derayea *et al.,* [2013] have reported spectrophotometric method for determination of five 1,4-dihydropyridine drugs using N-Bromosuccinimide and Indigo carmine dye by determining residual of NBS through its ability to bleach the colour of the used dye. Beer's law was obeyed in concentration range 1.25-13.00µg/ml. Good correlation coefficient was found between absorbance value and concentrations. Limit of detection ranged from 0.141 to 0.500µg/ml. This method was applied to the analysis of dosage forms, % recovery ranged from 97.31 to 99.4% without interference from common excipients. The comparision by student's t-test and variance ratio F-test showed no difference between the proposed and official methods.[19]

Mohamed Abo EI-Hamd *et al.,* [2010] have investigated spectrophotometric and spectrofluorimetric methods for determination of 1,4-dihydropyridine (1,4-DHP) drugs based on the oxidation of the investigated 1,4-DHP drugs with acidic $KMnO_4$(method I) or Ce (IV) (method II). First method was based on decrease in colour of permanganate solution due to the presence of the studied drug and measured at 525nm. Second method was based on monitoring the fluorescence of the produced cerium (III) at emission 355nm and excitation at 255nm. The method was validated according to International Conference of Hormonization guidelines. This

method was applied for the determination of drugs in commercial tablets and capsules. The result of the proposed procedures were statistically compared with reference method obtained.[20]

Y. Awad Uday *et al.,* [2011] have developed a simple, selective, rapid, precise, sensitive and accurate HPLC method for the estimation of nifedipine using methanol and water (70:30 v/v) as mobile phase; pH was adjusted to 3.0 with orthophosphoric acid. Detection was carried out using UV detector at 238 nm. The method was carried out on a Shim-Pack CLC, ODS (C-18), 5 µ column and dimension of column was 250×4.6mm. The retention time of nifedipine was 3.401 min. The developed method was validated in terms of linearity, precision, accuracy, precision, limit of detection and limit of quantitation. The method was found to be linear in the range of 5-40 µg/ml.[21]

Ravin Jugade *et al.,* [2011] have established A simple and rapid differential pulse polarographic method was developed for the trace determination of Nifedipine. A well-defined single peak with Ep value of -0.51 V was obtained in 0.1M acetate buffer (pH 5.0). The linearity was valid up to 5×10^{-5} M *(*r =0.9995) with minimum detection limit of 3.5×10^{-8}M. Precision of the method developed was implied from the values of relative mean deviation, standard deviation and coefficient of variation, which were 2.05%, 1.1 and 3.2% respectively. Marketed formulations of Nifedipine have been analyzed by calibration and standard addition methods. Recovery experiments have been found to be quantitative and analysis to determine the mass per tablet was obtained within ±0.2% of the expected value.[22]

Bengi Uslu *et al.,* [2010] have studied and reported a square wave voltammetry (SWV), which was presented in this mini review, had a several advantages such as high speed, increased analytical sensitivity and relative insensitivity to the presence of dissolved oxygen. Also it was an electrochemical technique used in analytical applications and fundamental studies of electrode mechanism. This paper delivered both the underlying theory and the practical guidance needed to apply square wave techniques and also provided a wide collection of data for the description of diverse tendencies that characterized several electrochemical reactions analyzed by SWV. This review summarized some of the recent developments and application of direct and stripping SWV for drug compounds in their dosage forms and biological samples.[23]

Dragica Zendelovska *et al.,* [2006] have studied a HPLC method for the determination of nifedipine in human plasma followed after solid-phase extraction. A selective, sensitive and accurate high-performance liquid chromatographic method has been developed, validated and applied for determination of nifedipine in human plasma samples. The method involved solid-phase extraction on C_{18} cartridges. The chromatographic separation was accomplished on a Lichrocart Lichrospher 60 RP select B column with a mobile phase composed of 0.020 mol/L KH_2PO_4 (pH 4.8) and acetonitrile (42:58, v/v). UV detection was set at 240 nm. The calibration curve was linear in the concentration range of 5.0–200.0 ng/mL for nifedipine in plasma and the limit of quantification was 5.0 ng/mL.[24]

Arindam Mukhopadhyay *et al.,* [2012] have studied LCMS/MS method for the determination of Nifedipine and Nifedipine D6. They were extracted from the plasma by precipitation method and then seperated on a reverse phase chromotography using the mobile phase mixture of ammonium acetate and methanol at a flow of 1.0 ml/min. The analytes were detected in API 4000 mass spectrometer in the positive atmospheric pressure chemical ionization (APCI) mode with mutiple reaction monitoring (MRM). The MRM transitions were monitored by following m/z for parent ion 347.2 & daughter ion 315.1 (nifedipine), and m/z 353.2 & daughter 318.2 (nifedipine D6). A linear calibration plot of nifedipine was achieved in the concentration ranges of 1.558 ng/ml to 360.561 ng/ml. Mean recovery was 93.2%. The assay was specific, precise, accurate and reproducible.[25]

Madhuri Ramteke *et al.,* [2010] have development an accurate and precise high performance thin layer chromatographic method for simultaneous estimation of atenolol and nifedipine in their combined dosage form was developed. The study employed Kieselghur 60, GF254 on aluminium foil and a mobile phase comprising cyclohexane: methanol: ethyl acetate: ammonia (5:1.5:3:0.5 v/v). The detection was carried out at 230 nm. The linear detector response for atenolol was observed between 5.7-18.9 mg/mL. The recovery study was carried out by standard addition method. The results of recovery were 99.76 ± 0.216, 100.72 ± 0.216 for atenolol and 100.04 ± 1.069, 99.89 ± 1.058 for nifedipine.[26]

Shubhini A. Saraf *et al.,* [2010] A rapid and sensitive RP-HPLC method has been developed for the purpose of analysis of antihypertensive: nifedipine (NF), antidiabetic: nateglinide (NG) and hypolipidemic: lovastain (LT) drugs simultaneously in cardiovascular polypill based synthetic ternary mixture. Analysis was performed on C_{18} (125 4.6 mm id, 5 m particle) column with acetonitrile- 10mM phosphate buffer (pH 3.5) 60:40 (v/v) as mobile phase, started at a flow rate of 1 mL min^{-1} continued for 4 min and further 6 min at a flow rate of 2 mL min^{-1}. UV detection was performed at 208 nm for NF, NG and at 236 nm for LT. The run time under these chromatographic conditions was less than 10 min. The method was linear in the range of 0.125-8.0 $mg\ mL^{-1}$ for NG and LT. Acceptable precision and accuracy were obtained for concentrations over the standard curve ranges. The sensitivity of the method, as the limits of detection (LOD) and quantification (LOQ) for each active ingredient was also determined. The validated method was successfully applied to the analysis of synthetic mixture and of three drugs. The percentage recoveries obtained were 100.23% for NF, and 100.93% for LT.[27]

Hisham S. Abou-Auda *et al.,* [1999] have studied sensitive, selective and reproducible reversed-phase high-performance liquid chromatographic method was developed for the determination of nifedipine in human plasma with minimum sample preparation. The method was sensitive to 3 ng/ml in plasma, with acceptable within and between day reproducibilities and linearity (r^2>0.99) over a concentration range from 10-200 ng/ml. Acidified plasma samples were extracted using diethylether containing diazepam as internal standard and chromatographic separation was accomplished on C_{18} column using a mobile phase consisting of acetonitrile, methanol and water (35:17:48, v/v). The within-day precision ranged from 2.22 to 4.64% and accuracy ranged from 102.4-106.4%. The day-to-day precision ranged from 2.34-7.07% and accuracy from 95.1-100.1%. The relative recoveries of nifedipine from plasma ranged 91.0-107.3% whereas extraction recoveries were 88.6-93.3 %. Following eight 6-week freeze-thaw cycles, nifedipine in plasma samples proved to be stable with accuracy ranging from 0.64 to 3.0 % and precision ranging from 3.6 to 4.15%. Nifedipine was also found to be photostable for at least 120 min in plasma, 30 min in blood and for 60 min in aqueous solutions after exposure to light. The method was sensitive and reliable for pharmacokinetic studies and therapeutic drug monitoring of

nifedipine in humans after the oral administration of immediate-release capsules and sustained-release tablets to five healthy subjects.[28]

M.L. Avramov Ivic *et al.,* [2013] have examined the electrochemical behaviour of inclusion complexes of nifedipine (Nif) and amlodipine (Aml), a long-acting calcium channel blockers dihydropyridine (DHP) class, with -cyclodextrin (CD) and (2-hydroxypropyl)- -cyclodextrin (HP CD), was examined using cyclic and square wave voltammetry in 0.05 M $NaHCO_3$ and phosphate buffer (pH=11) on a gold electrode. The voltammograms showed a single irreversible anodic wave with the current controlled by adsorption. It was found that phosphate buffer was favourable in the electrochemical activity of both complexes of Nif with the linear dependency of the oxidative currents on their concentrations. In phosphate buffer, only HP CD-Aml complex showed linear dependency of the oxidative apparent currents maximum of all the examined complexes in both electrolytes were shifted to the positive direction compared to their standards. In addition, the value of anodic currents decreased.[29]

Albetro S. Pereira *et al.,* [2008] have analyzed the fragmentation of dihydropyridine calcium-channel antagonists were compared by electrospray ionization (ESI) and atmospheric pressure photonization (APPI). The results demonstrated that in ESI the preferred ionization process is in positive mode, with the mass spectra of $[M+H]^+$ showing base peak ions probably formed by loss of alcohols from carboxyl groups. Conversely, in APPI, a high intense peak is observed in negative mode due to deprotonated molecule $[M+H]^-$ after two serial 1,2-hydride shifts leading to a rearranged deprotonated molecule $[M-H]^-$. These ions suffered another 1,2-hydride shifts to produce a nitro-phenyl product ion of m/z 122. The APPI was also used to develop a method for the quantitation of dihydropyridines (e.g., nifedipine) in human plasma.[30]

Morteza Pirali *et al.,* [1999] have performed a experiment using a simple normal phase high performance liquid chromatography (HPLC) method for determination of nifedipine in human plasma. The method was based on ultraviolet detection at 235 nm and acidic plasma extraction by a mixture of dichloromethane (30%) and n-hexane (70%) using nimodipine as an internal standard. The system was stabilized with the use of n-hexane (80%), chloroform (17%) and methanol (3%) as mobile phase. The assay was linear for 3 ng/ml plasma. The reproducibility of

the method was satisfactory. The procedure could be used effectively to quantitative in the drug.[31]

Luis J. Nunez-Vergara *et al.,* [2002] have studied a gas chromatography/mass spectrometry (GC/MS) method for the qualitative and quantitative determination of the calcium-channel anatagonists C-4-substituted 1,4-dihydropyridines and their corresponding N-ethyl derivatives, was presented. Also, the electrochemical oxidation and the reactivity of the compounds with alkyl radicals derived from 2,2-azobis-(2-amidinopropane) were monitored by GC/MS. Mass spectral fragmentation patterns for the C-4-substituted 1,4-dihydropyridine parent drugs were significantly different from those of their oxidation products, generated either by electrochemical oxidation or by reaction with alkyl radicals. However, for N-ethyl-1,4-dihydropyridine compounds it was not possible to detect the final products (pyridinium salts) using these experimental conditions.[32]

R.L.C. Sasidhar *et al.,* [2012] have developed a simple precise and economical reverse phase high performance liquid chromatographic method for the estimation of atenolol and nifedipine simultaneously in combined dosage form. The method was developed using agilent ODS C_{18} column with a mobile phase constituting of methanol: acetonitrile: phosphate buffer (60:20:20) finally adjusted to pH 3.0 with o-phosporic acid at a flow rate of 1.0 ml/min and detection was carried out at 235 nm. The selected chromatographic conditions were found to separate atenolol (Rt: 2.80 min) and nifedipine (Rt: 4.40 min) having a resolution of 12.307. The developed method was validated for linearity, accuracy, precision, LOD, LOQ, robustness and for system suitability parameters as per ICH guidelines. Linearity for atenolol and nifedipine were found in the range of 5-25 ig/ml and 2-10 ig/ml, respectively. The percentage recoveries for atenolol and nifedipine ranged from 99.38-100.56% and 99.16 - 99.71%, respectively. The proposed method could be used for routine analysis of atenolol and nifedipine in their combined dosage forms.[33]

V. Venkata Rajesham *et al.,* [2008] have developed a simple and sensitive high performance liquid chromatographic method for the simultaneous estimation of simvastatin (lipid lowering agent) and nifedipine (calcium channel blocker) in rat plasma and also to calculate possible pharmacokinetic parameters of these drugs after oral administration in hyperlipidemic condition. The plasma samples were precipitated with methanol and after centrifugation (5000-6000 rpm for 10 min), the acetonitrile was added to the ethanol and the organic mixture was taken to near dryness by a steam of nitrogen at room temperature. The mobile phase consisted of acetonitrile

and water. The drug was monitored at 237nm. The run time was 15 minutes. The good linearity was found to be in the range of 0.05 to 40 mg/ml for two drugs. This developed method was rapid, sensitive, reproducible and successfully applied to the measurement of simvastain and nifedipine in rat plasma for studying of pharmacokinetic interaction between these two drugs.[34]

XingJie Guo *et al.,* [2007] have determined a sensitive atmospheric pressure chemical ionization liquid chromatographic-mass spectrometric (APCI-LC-MS) assay with positive ion mode was developed for the determination of nifedipine in human plasma. In this method, nifedipine was extracted from human plasma using diethyl ether with dimethoxanate as the internal standard. Analysis was achieved on a BDS C_{18} column with methanol-water (66:34, v/v) as the mobile phase. Sustained-release nifedipine tablets from Disha (test, Weihai, China) from GuoFeng (reference, Qingdao, China) were evaluated following a single 20 mg oral dose to 20 healthy volunteers. Bioequivalance between the products was determined by calculating 90% confidence intervals (90% CI) for the ratio of C_{max} , AUC_{0-t} and $AUC_{0-¥}$ values for the test and reference products, using logarthmic transformed data. The 90% confidence intervals for the ratio of C_{max} (86.6 - 105.2%), AUC_{0-t} (97.8 - 110.9%) and $AUC_{0-¥}$ (96.5 - 110.4%) values for the test and reference products were within the interval (80 - 125% for AUC, and 70 - 143% for C_{max}) proposed by state of food and drug administration [SFDA, 2005 guidence for bioavailability studies for chemical drug products in human being, China, p.19]. It was concluded that the two sustained-release nifedipine tablets are bioequivalent in their rate and extent of absorption and thus may be used interchangeably.[35]

C.M. Andersen *et al.,* [2003] have expreimented a dedicated investigation and pratical description of how to apply PARAFAC modelling to complicated fluorescence excitation-emission measurements. The steps involved in finding the optical PARAFAC model were described in detail based on the characteristics of fluorescence data. These steps included choosing the right number of components, handling problems with missing values and scatter, detecting variables influenced by noise and identifying outliers. Various validation methods were applied in order to ensure that the optimal model has been found and several common data-

specific problems and their solutions were explained. Finally, interpretations of the specific models were given. The paper could be used as a tutorial for investigating fluorescence landscapes with multi-way analysis.[36]

INTRODUCTION TO PRESENT STUDY

UV-Visible spectrophotometric method and quenchofluorimetric methods were adopted for present work for the quantification of nifedipine in tablet formulation.

UV-Visible spectrophotometric determination of nifedipine:

On the examination of the chemical structure of nifedipine revealed the presence of aromatic rings with $–NHCOCH_3$ and nitro substituent which are strongly electron withdrawing groups. Nifedipine as expected from its structure, exhibits light absorbing property in UV-Visible region having λ-max at 330 nm in methanolic solution which was made use for its quantitative method by absorptiometric method.

Quenchofluorimetric determination of nifedipine:

Nifedipine did not exhibit any native fluorescence because of the strong electron withdrawing substituents. Therefore the drug was examined whether it has any quenching property.

A number of fluorescent molecules were tried as fluorescent probe (donor). Quinine sulphate, N-1 Napthyl Ethylene Diamine, and fluorescein were the fluorescent probes tried. Among them, the reagent `Quinine sulphate' was found to be more suitable because of their similarity in the solubility of drug and the donor in the same media methanol. The fluorescent intensity of quinine sulphate was stable and maximum in methanol. Hence this probe moiety was selected.

First the linear relationship was established between concentration of quinine sulphate and fluorescence intensity. From this, the optimum concentration of quinine sulphate solution was selected for the quenchofluorimetric method. The nifedipine standard solution and test solution were prepared. Then mixture of quinine sulphate and nifedipine, with quinine sulphate concentration fixed and the concentration of nifedipine were varied. The fluorescence intensity for the mixture were measured at the characteristic excitation wavelength. The fluorescence intensity diminishes linearly for the quinine sulphate with the increasing concentration of nifedipine. The quenching caused by known concentration of the drug (Nifedipine) was compared with that produced by test preparation.

The characteristics of Quinine sulphate was briefly given below,

QUININE SULPHATE

It was obtained from the bark of various species of cinchona including succirubra, pavon red cinchona officinalis, Linn

Chemical Structure :

Molecular Weight	: 782.96
Molecular Formula	: $(C_{20}H_{24}N_2O_4)_2.H_2SO_4.2H_2O$
Melting point	: 235 ^{O}C
Description	: Fine, white, needle like crystals, odorless, darkens on exposure to light.
Solubility	: Slightly soluble in water Slightly soluble in ethanol and in boiling water Practically insoluble in ether

Use of quinine sulphate : It is used in the treatment of malaria, leg cramps, and used to treat falciparum malaria.

Light absorption property : Quinine Sulphate is a light absorbing as well as a fluorescent compound. It exhibits light absorption in the range of 200 nm - 400 nm in methanol with the maxima at 280 nm and 334 nm. The fluorescence spectrum of quinine has excitation maxima at 350 nm and emission maxima at 450 nm.

AIM OF PRESENT STUDY

Nifedipine is an anti hypertensive drug. Nifedipine is official in IP, BP, and USP. Determination of nifedipine raw material in I.P is based on HPLC separation on ODS (5 µm) with mobile phase 55:36:9 volumes of water, methanol and acetonitrile and detected at 235nm. The finished product formulation have been quantitated by UV spectrophotometric method at 350 nm and using methanol as medium.

Literature review was made thoroughly on the above drug. Different analytical methods have been reported such as UV-Vis spectrophotometry, RP-HPLC, HPTLC, LC-MS, and GC/MS and polarographic methods for the determination of nifedipine in its dosage form and in combination with other drugs and in biological matrix.

The present work is the development of modified quenchofluorimetric method. The determination of nifedipine has been made by an indirect fluorimetric method based on its quenching ability upon an fluorescent probe. In this technique a suitable fluorescent probe moiety quinine have been tried to determine the drug. The method utilizes decrease of the fluorescence intensity of the probe moiety by the selected drug. The linearity exists between the concentrations of the active drug to the degree of fluorescence quenched upon the probe moiety quinine sulphate .

To arrive at the accuracy of the proposed method, its assay result was compared with that obtained by UV-Visible spectrophotometric method. The later method involved measurement of absorbance of the drug nifedipine in methanol at its λ_{max}. The assay method has been validated.

METHODOLOGY

Quenchofluorimetric determination of nifedipine using Quinine Sulphate as a probe moiety :

Apparatus/ Instruments used :

Spectrofluorimeter RF - 5301 PC

Analytical electronic weighing balance (Shimadzu)

Chemical used :

Sulphuric acid AR grade

Distilled water

Methanol HPLC grade

Tablet Formulation :

Nifedipine Tablets - 10 mg (Alkam)

Brand name - Nifedine

METHOD DEVELOPMENT

Establishment of various parameters for the drug nifedipine :

Selection of excitation and emission maxima :

Preparation of 0.05M H_2SO_4 :

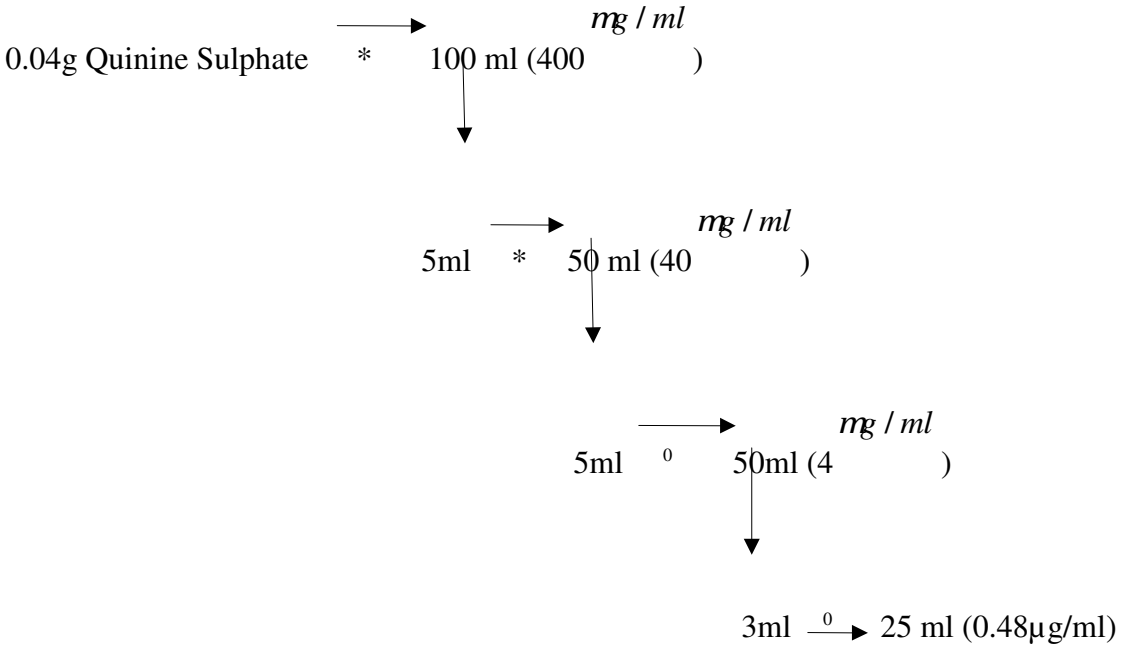
2.8 ml of H_2SO_4 was diluted to 1000 ml with distilled water to produce 0.05M H_2SO_4.

Preparation of Quinine Sulphate Solution :

0.04g of Quinine Sulphalte was accurately weighed and transferred into 100 ml of standard flask. The sample was dissolved in small volume of 0.05 M H_2SO_4 and the volume was made up to 100 ml (400 mg / ml). 5 ml of this solution was pipetted into 50 ml standard flask and diluted with 0.05 M H_2SO_4 (40 µg / ml) From this 5 ml of the solution was

pipetted into 50 ml standard flask and diluted with methanol (4 *mg / ml*). From this, 3 ml of the solution was diluted to 25ml with methanol. The concentration of quinine in final dilution was 0.48 µg / ml. This solution was used to obtain the fluorescence excitation and fluorescence emission spectrum of quinine sulphate.

Dilution chart of Quinine Sulphate Solution :



* → 0.05M H_2SO_4 ,0- methanol

Excitation and emission spectrum of Quinine Sulphate :

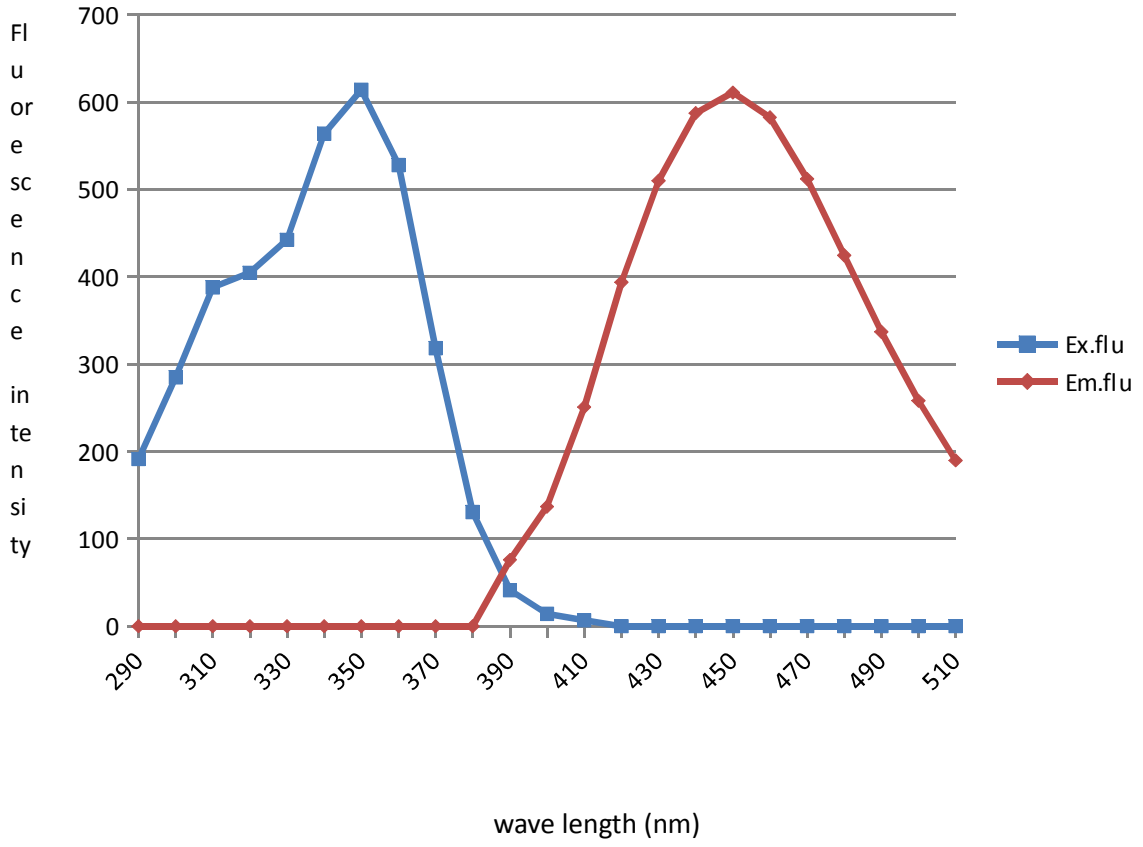
Quinine Sulphate solution was prepared with methanol to get a final concentration of 0.48 *mg / ml*. The solution was used to scan in the wavelength range of 290 - 410 nm for excitation and 390 - 510 nm for emission spectrum. From the spectrum of Quinine Sulphate, the optimum wavelength for excitation and emission were selected and found to be at 350 nm and 450 nm respectively. The data for excitation and emission spectrum are presented in table number 1,

Table no 1 Data for Fluorescence excitation and emission spectrum of Quinine Sulphate

Wavelength for Excitation (nm)	**Fluorescence Intensity**	**Wavelength for emission (nm)**	**Fluorescence Intensity**
290	191.006	390	75.801
300	284.697	400	136.663
310	387.656	410	250.872
320	404.506	420	393.603
330	442.202	430	509.555
340	563.573	440	587.188
350	**613.878**	**450**	**610.778**
360	527.667	460	582.284
370	318.106	470	511.851
380	130.567	480	424.246
390	40.985	490	336.846
400	14.039	500	257.921

410	6.732	510	189.657

Graph no 1 Fluorescence excitation and emission spectra for Quinine Sulphate



From the spectra it was found that excitation maxima is at 350 nm and emission maxima is at 450 nm.

Selection of pH :

A stock solution of Quinine Sulphate was prepared in methanol with a concentration of 400 µg /ml. This was stepwise diluted in methanol to get a concentration of 4 µg/ml. 3ml of this solution was diluted with different medium.

Dilution chart :

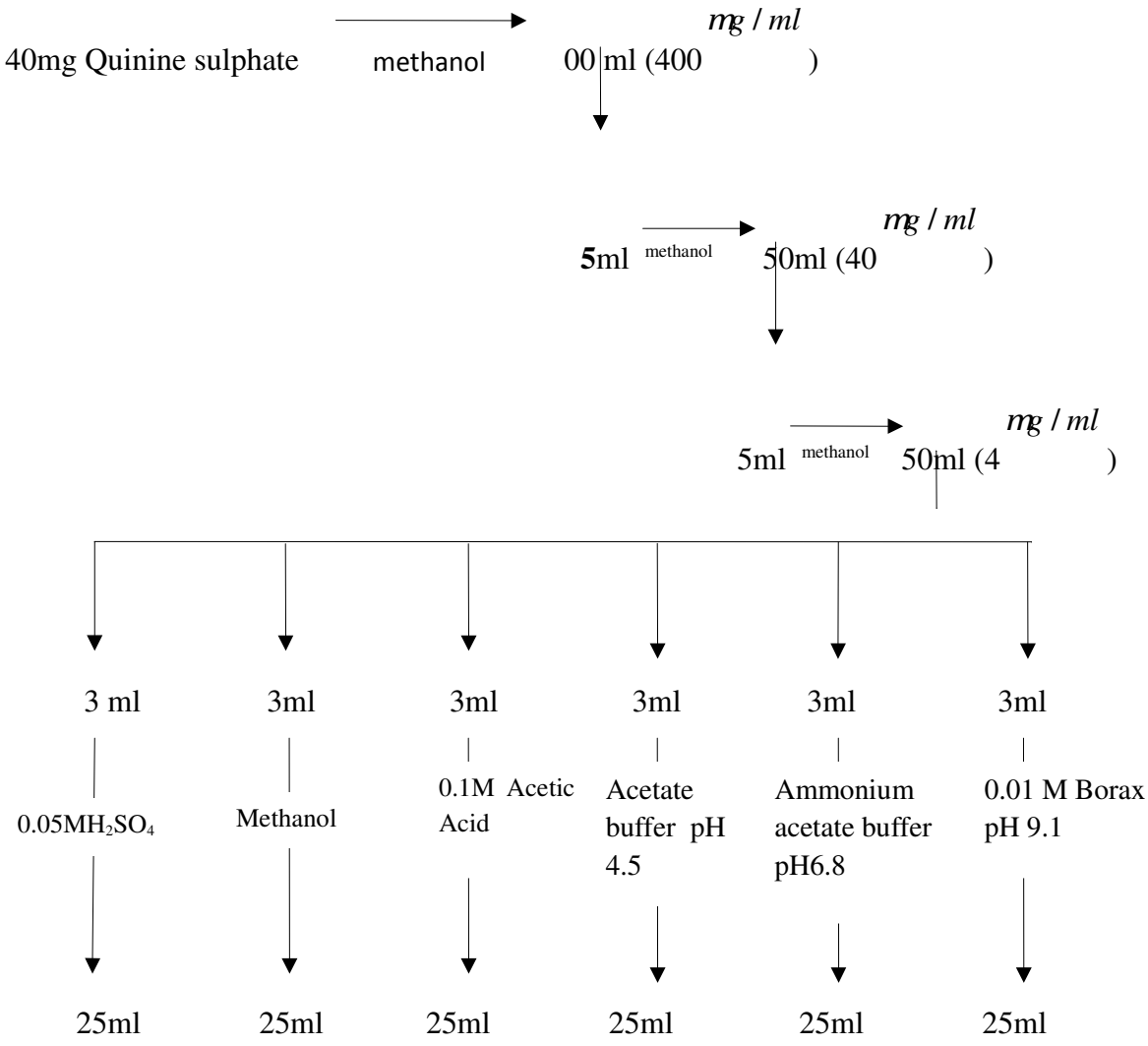


Table no 2 Data for effect of pH upon fluorescence

Probe Moiety	Excitation wavelength (nm)	Emission wavelength (nm)	Reagents	F.I
			0.05 M H_2So_4	924
			Methanol	576

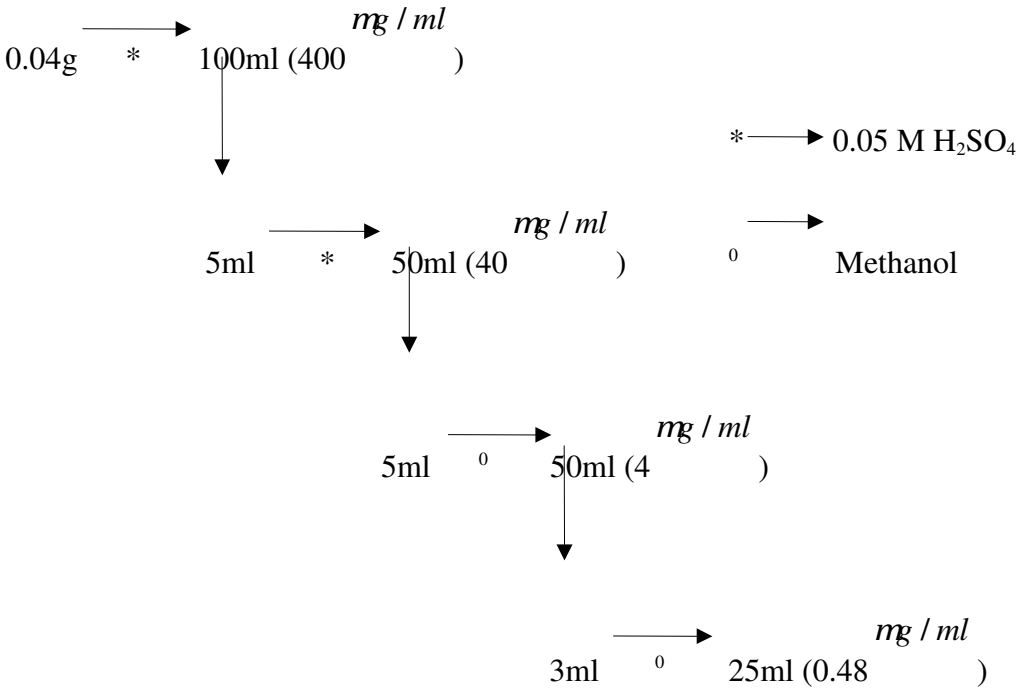
Quinine Sulphate *mg / ml* (0.48)	350	450	0.1M acetic acid (pH-2.8)	676
			Acetate buffer (pH-4.5)	550
			Ammonium acetate buffer (pH-6.8)	530
			0.01 M Borax(pH-9)	50

The Fluorescence Intensity was maximum in 0.05M H_2SO_4.

Effect of temperature upon Fluorescence Intensity :

0.04g of Quinine sulphate was accurately weighed and transferred into a 100 ml standard volumetric flask and made up with0.05 M H_2SO_4 acid. From this 5 ml was pipetted and transferred into a 50 ml standard volumetric flask and made up with 0.05 M H_2SO_4. Further dilution of 5 ml was pipetted out and transferred into 50 ml standard volumetric flask and made up with methanol. The final concentration of Quinine sulphate was found to be *mg / ml* 0.48 . The effect of temperature upon Fluorescence Intensity was determined by maintaining the last solution at different temperatures such as 15ºC, 20ºC, 25º, 30ºC & 35ºC.

Dilution chart :



Data for effect of temperature upon Fluorescence Intensity :

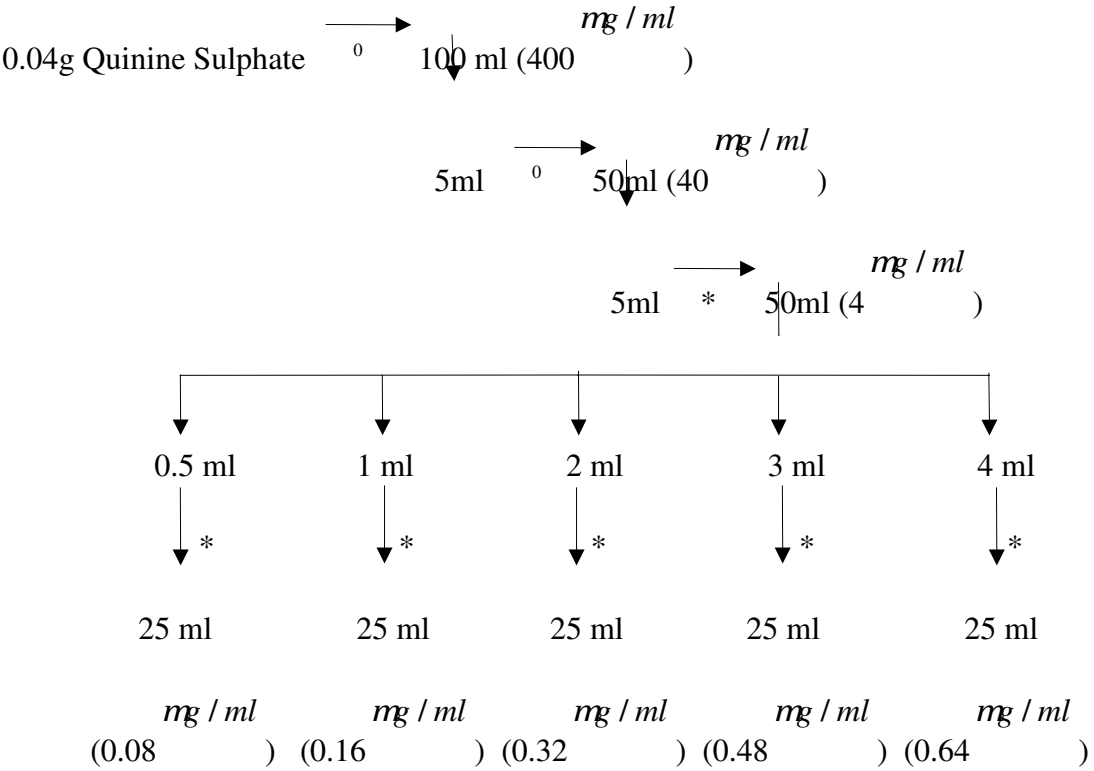
Table no 3

Probe Moiety	Ex. Wavelength (nm)	Em. Wavelength (nm)	Temperature (°C)	FI
Quinine sulphate mg / ml (0.48)	350	450	15	575.464
			20	570.293
			25	563.512
			30	550.493

Construction of analytical curve for Quinine Sulphate :

A stock solution of Quinine Sulphate with the concentration of 400 µg /ml was prepared in methanol . From this, the solution was stepwise diluted to obtain a series of standard solution ranging in the concentration of 0.08 µg/ml to 0.64 µg/ml. The final dilution was made with methanol as medium. The samples were excited at the wavelength of 350 nm and fluorescence emission measured at 450 nm . The calibration curve was constructed by plotting concentration of Quinine Sulphate versus fluorescence intensity. The data for standard curve has been given in table no 2.

Dilution Chart :



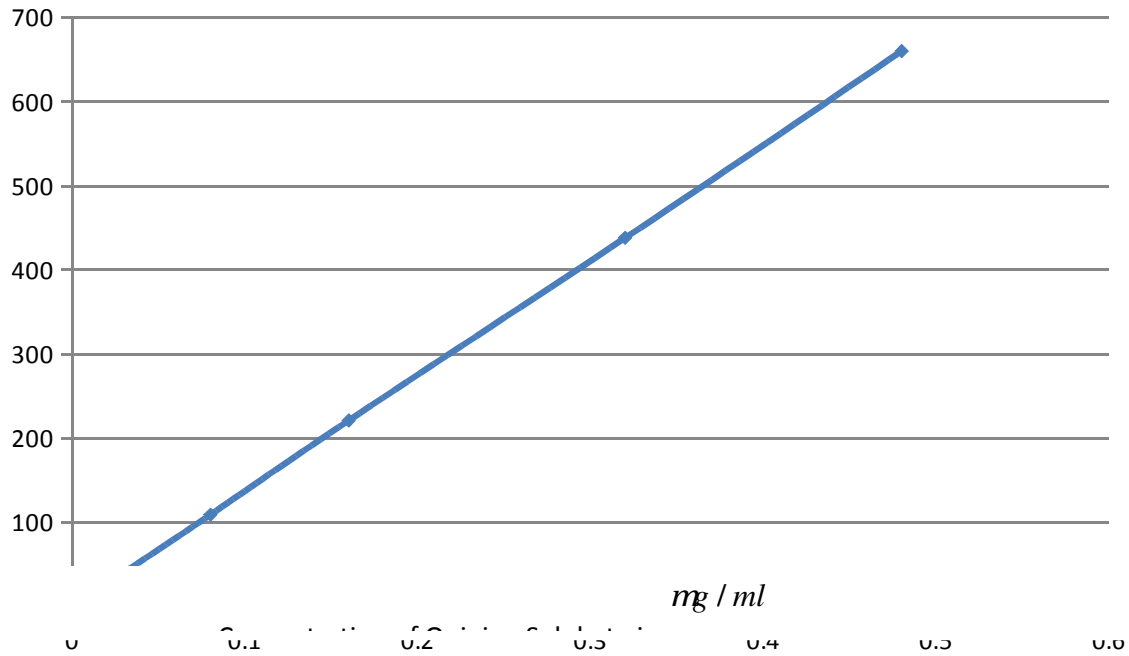
* → methanol

0 → 0.05 M H_2SO_4

Table no 4 Data for calibration curve of Quinine Sulphate using Methanol as medium

S. No	Concentration (µg/ml)	Fluorescence Intensity
1	0.08	109
2	0.16	221
3	0.32	438
4	0.48	660
5	0.64	876

Graph no 2 Calibration curve of Quinine Sulphate



Correlation Coefficient = 0.999989

LINEARITY :

Procedure for study of quenching property of Nifedipine :

A calibration curve for the study of quenching by Nifedipine was constructed by adopting the following steps.

Nifedipine has been shown to posses quenching property upon Quinine Sulphate. The degree of quenching by the drug nifedipine upon the fluorescence of Quinine Sulphate

was studied by preparing a series of concentration of nifedipine with constant amount of Quinine Sulphate.

A stock solution of nifedipine was prepared in methanol (1000 µg / ml). The stock solution was diluted ten times with methanol to obtain the concentration of 100 µg / ml . From this, different volume of the solution 0 to 5ml were transferred into different 25ml standard volumetric flask . To each flask, 3ml of Quinine Sulphate solution (4 µg / ml) was added. Each mixture was diluted to the mark with methanol. After the final dilution , each flask contain constant concentration of Quinine Sulphate of 0.48 µg / ml and the drug nifedipine ranges from 0 to 20 µg / ml.

The difference in the fluorescence intensity between pure quinine solution and that of mixture containing Quinine and nifedipine was taken as the amount of quenching.

Dilution chart for fluorescence quenching by Nifedipine :

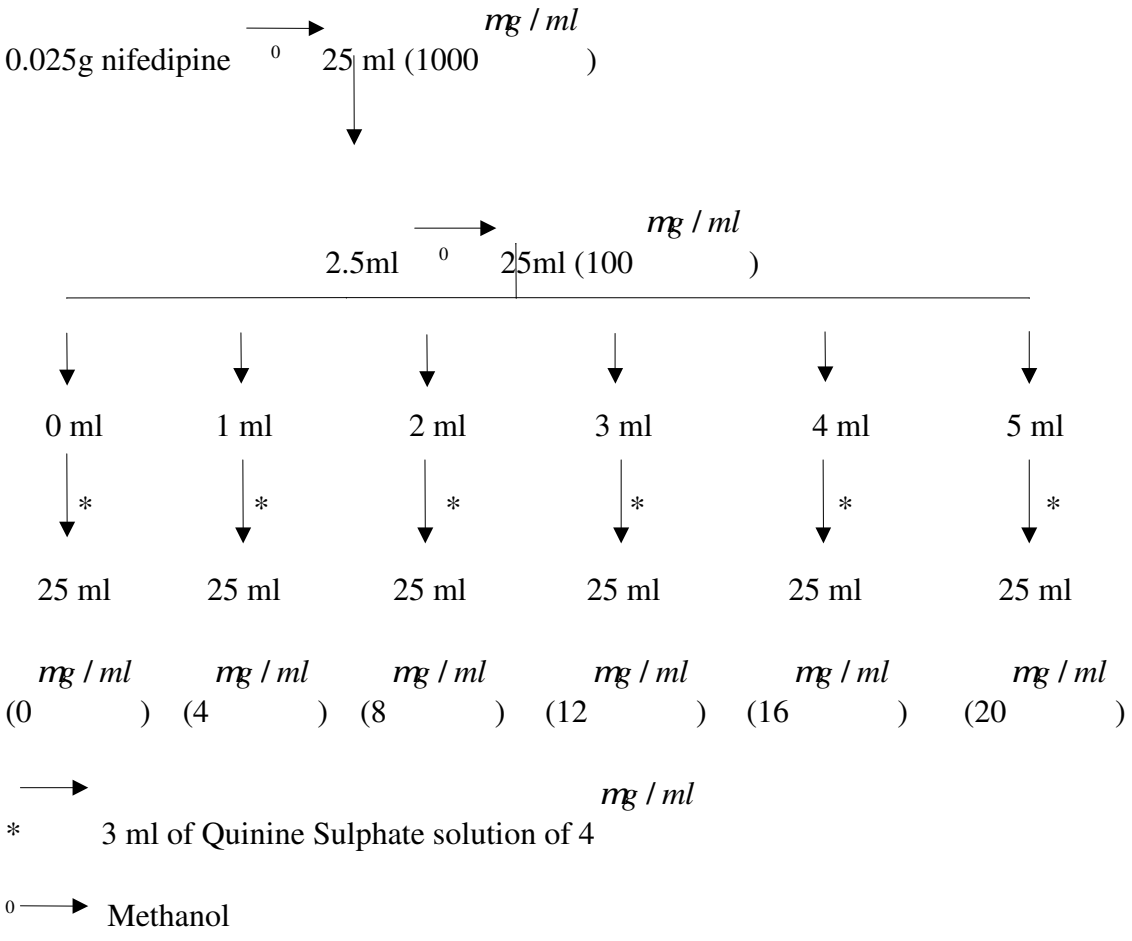
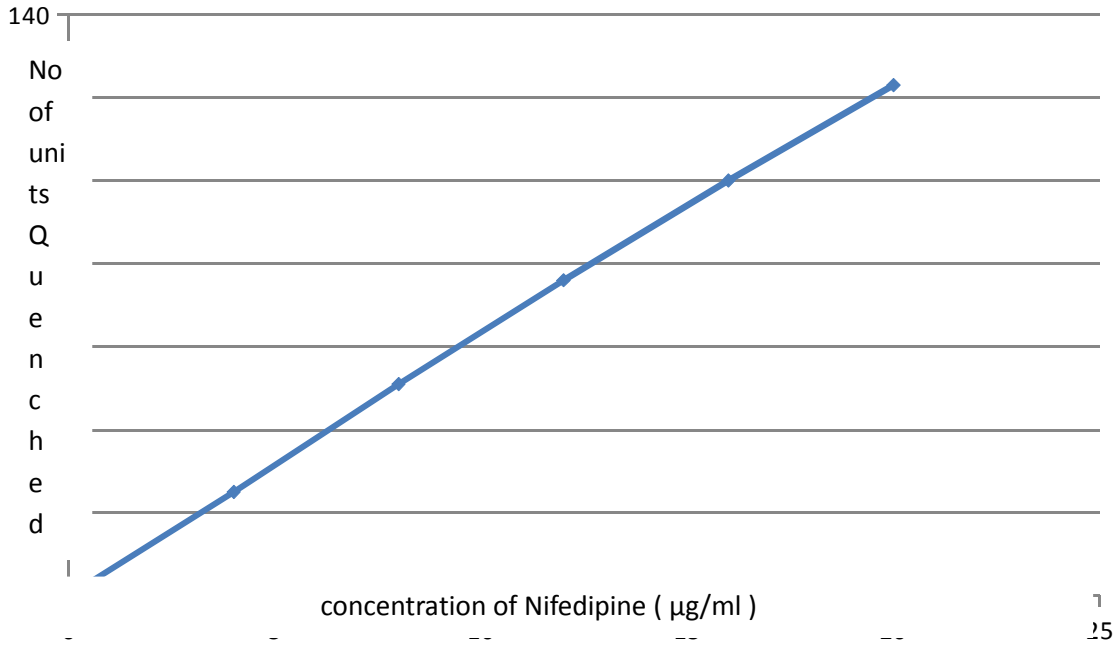


Table no 5 Data for calibration curve for Nifedipine

S. No	Concentration of Nifedipine *mg / ml* ( )	No. of units quenched
1	4	25
2	8	51
3	12	76
4	16	100
5	20	125

Graph no 3 calibration curve for Nifedipine



Correlation Coefficient = 0.9999113

Limit of detection (LOD) :

The limit of detection an individual analytical procedure is the lowest amount of analyte in the sample which can detected but not necessarily quantitated as an exact value. The limit of detection (LOD) may be expressed as;

$$LOD = \sigma\ 3.3 / S$$

Where
σ = the standard deviation of the response
S = the slope of the calibration curve of the analyte

Limit of quantitation (LOQ) :

The limit of quantitation of an analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy.

Limit of quantitation (LOQ) can be expressed as;

$$LOQ = \sigma 10 / S$$

Where,
σ = standard deviation of the response
S = slope of the calibration curve of the analyte

Table no 6 Data for LOD and LOQ

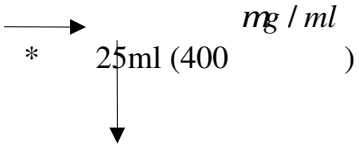
S. No	Name of the drug	LOD	LOQ
1	Nifedipine	0.03083	0.093449

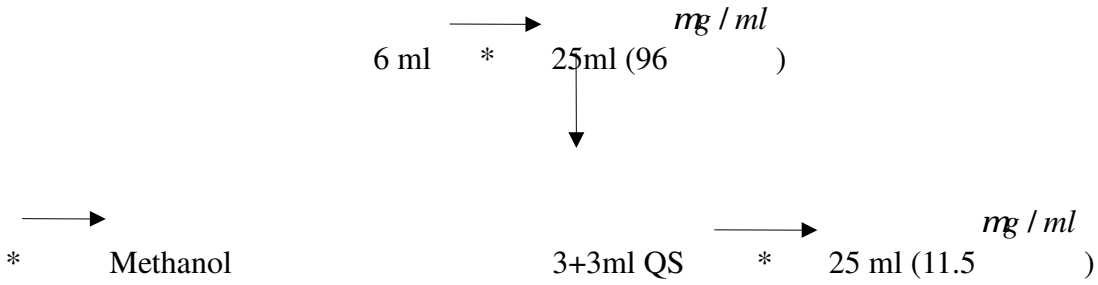
Assay :

Preparation of standard Nifedipine solution :

A standard solution of pure nifedipine with the concentration of 400 µg/ml was prepared in methanol. From this, 6ml of the solution was pipetted into 25ml standard volumetric flask, and the volume was made up with methanol. 3ml of the solution was pipetted from the above solution into another 25ml standard flask. To this 3ml of Quinine Sulphate solution was added. The mixture was made up to 25ml with methanol.

Dilution chart for standard Nifedipine solution :



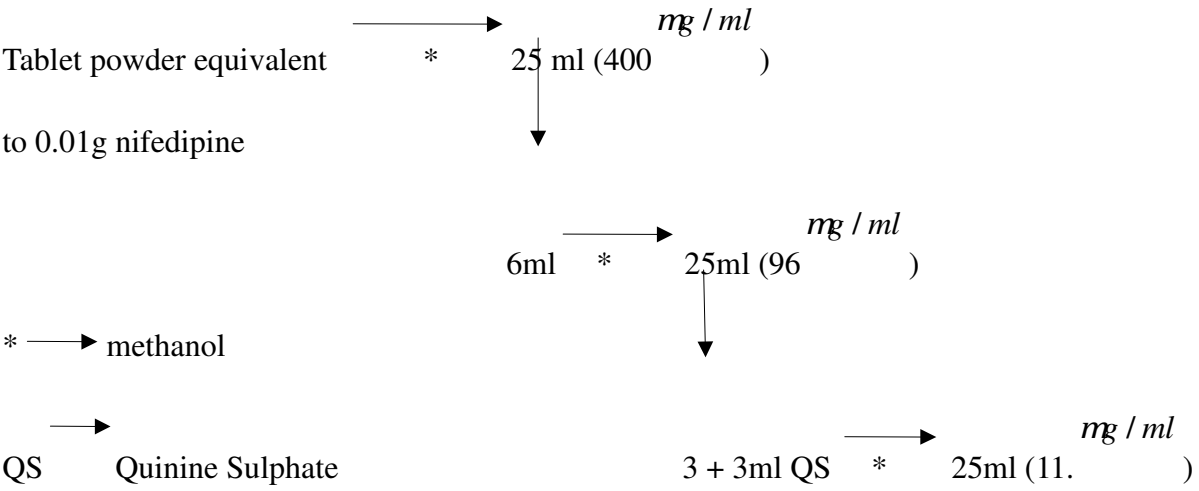


Preparation of test solution of Nifedipine :

Average weight of the tablet was computed from the weight of 20 tablets .The tablets were powdered in a mortor and pestle.

An amount of tablet powder was accurately weighed so as to contain 0.01g of pure nifedipine, transferred to a 25ml standard volumetric flask. The active ingredients present in the tablet was extracted by shaking the powder in 15ml of methanol for about 15minutes. The volume was made up with more methanol and mixed well. The solution was clarified by filtration. 6ml of clear filtrate was diluted to 25ml with methanol. 3ml of this solution was pipetted in to a 25ml standard flask. To this, 3ml of quinine sulphate solution (4 µg/ml) was added. The mixture was made up to 25ml.

Dilution chart for test solution of Nifedipine :



Preparation of control solution :

A control solution was prepared by omitting the drug and diluting 3ml of Quinine Sulphate solution to 25ml with methanol. The fluorescence intensity was measured by exciting the solution at 350 nm and measuring fluorescence emission at 450 nm.

Dilution chart for control :

3 ml of Quinine Sulphate $\xrightarrow{*}$ 25 ml

$* \longrightarrow$ methanol

The instrument was set at excitation wavelength 350 nm and emission wavelength at 450 nm. The fluorescence was measured for the standard, test and control solution.

The data of assay of nifedipine tablet is given in table no 4

Assay calculation :

The content of drug present in each tablet and average weight calculated by using following formula.

$$\frac{\text{Fluorescence quenched by test}}{\text{Fluorescence quenched by standard}} \times \text{wt of std} \times \text{dilution factor} \times \frac{\text{avg wt of tablet}}{\text{wt of test}}$$

Table no 7 Data for assay of tablet

Brand name - Nifedine

S. No	Wt of std (g)	FI Quenched	Wt of tablet powder (g)	FI Quenched	Content of nifedipine (g)
1	0.025	74	0.2303	73.2	0.01016
2			0.2302	73.4	0.01019
3			0.2310	73.0	0.01014
4			0.2305	72.8	0.01011
5			0.2300	72.6	0.01008

% RSD = 0.64 %

Interference studies :

The excipients most likely present in the tablet formulation were used to know its fluoresceing nature and quenching ability. Each of the commonly used excipients are talc, lactose and magnesium stearate and they were taken in the same proportion as found in tablet formula. First the extract of the excipients was separately prepared in methanol. After clarification by filtration, it was examined for their fluorescence character. Then the effect of quenching upon the probe moiety Quinine Sulphate was also studied by carrying out the experiment similar to that of assay of the formula.

The result for interference study is given in table no 8

Table no 8 Data for interference studies

S. No	Probe moiety	Probe Moiety with excipients	FI. Quenched
1	Quinine Sulphate	QS + talc	Nil
2		QS + lactose	Nil
3		QS+ magnesium Sterate	Nil

From the above result, we clearly know that the excipients do not posses fluorescence or they quench the fluorescence of Quinine sulphate.

Recovery Studies :

Procedure :

Recovery studies are performed to know that the experimental procedure is free from interference. Recovery studies were carried out at three different spiking levels namely 50%, 100%, and 150%. The active ingredient in tablet powder, spiked with pure drug was extracted with small volume of methanol and the volume was made up to 25ml and it was stepwise diluted with methanol and in the final dilution 3ml of quinine sulphate was incorporated and diluted to 25ml. The experimental procedure for the recovery studies is similar to that of assay procedure for tablets. To compute the % of recovery a standard solution of nifedipine

was also prepared. The degree of quenching was measured for the blank, standard preparation as well as the test preparation containing nifedipine present in the formulation spiked with known amount of nifedipine. The concentration of quinine sulphate in each of final dilution at each spiked level is 0.48 µg/ml. The blank solution containing quinine with 0.48 µg/ml was also prepared. A standard solution of nifedipine was prepared and the concentration of nifedipine in final solution was 11.5 µg/ml.

The scheme of dilution for three different spiking levels are shown in following tables,

Dilution chart for Recovery studies :

a) Dilution for standard :

0.015 g of nifedipine * 25 ml

↓

4 ml * 25ml

↓

3+3ml QS * 25ml (Conc. of NF 11.5µg/ml)

b) Spiking at 50% level

0.23g tablet powder
(equivalent to 0.01g) +
0.005g of spiked pure nifedipine * 25 ml

↓

4ml * 25ml

↓

3+3ml QS * 25ml
(Total NF 11.5µg/ml)

c) Spiking at 100% level

0.23g tablet powder
(equivalent to 0.01g) +
0.01g of spiked pure nifedipine * → 25ml

↓

3ml * → 25ml

↓

3+3ml QS * → 25ml

(Total NF 11.5µg/ml)

d) Spiking at 150% level

0.115 g tablet powder
(equivalent to 0.005g) +
0.01g of spiked pure Nifedipine * → 25ml

↓

4ml * → 25ml

↓

3+3ml QS * → 25ml

(Total NF 11.5µg/ml)

* → methanol

QS → Quinine Sulphate solution (0.48 µg/ml)

Recovery studies for Nifedipine

Table no 9 Data for recovery studies at 50 % level

S. No	Wt of std (g)	Conc. of std NF (µg/ml)	FI Quenched	Wt of tablet powder (g)	Amount of NF spiked (g)	Total conc after spiking	FI Quenched	% Recovery
1				0.2302			74	100%

2	0.015	11.5	74	0.2303	0.005	11.5	73	98.6%
3				0.2300			74	100%
4				0.2305			73.1	98.78%
5				0.2300			73	98.6%

Table no 10 Data for recovery studies at 100 % level

S. No	Wt of std (g)	Conc. of std NF (µg/ml)	FI Quenched	Wt of tablet powder (g)	Amount of NF spiked (g)	Total conc after spiking	FI Quenched	% Recovery
1	0.015	11.5	74	0.2301	0.01	11.5	72.7	98.24%
2				0.2309			72.8	98.37%
3				0.2300			74.0	100%
4				0.2308			73.0	98.64%
5				0.2306			72.7	98.24%

Table no 11 Data for recovery studies at 150 % level

S. No	Wt of std (g)	Conc. of std NF (µg/ml)	FI Quenched	Wt of tablet powder (g)	Amount of NF spiked (g)	Total conc after spiking	FI Quenched	% Recovery
1	0.015	11.5	74	0.1154	0.01	11.5	73.4	98.24%
2				0.1154			73.0	98.37%
3				0.1159			73.4	100%
4				0.1150			73.0	98.64%
5				0.1155			73.5	98.24%

ROBUSTNESS :

The Robustness is a measure of method capacity to remain unaffected by small, deliberate variations in method parameters and provides an indication of method reliability during normal use.

Change of Analytical Excitation and Emission wavelength :

The standard solution, control solution and test solution of Nifedipine were prepared as per the tablet assay procedure. The degree of quenching was measured at 3 different sets of excitation and emission wavelength and the content of tablet powder was determined. The data for the study on robustness has been given in the table no 9. Assay of tablet was done at the analytical wavelength 350 nm as excitation, 450 nm as emission. The analytical wavelength was changed towards shorter and longer wavelength. The analytical wavelength were shifted at ± 5nm for excitation and emission wavelength and % RSD was calculated.

Data for Assay of Nifedipine tablet at different wavelengths

Excitation wavelength- 350 nm and Emission wavelength- 450 nm

Table no 12 Data for assay of nifedipine at analytical wavelength

S. N o	Wt of std (g)	FI Quenched	Wt of tablet powder (g)	FI Quenched	Content of nifedipine(g)
1	0.025	74	0.2303	73.2	0.01016
2			0.2302	73.4	0.01019
3			0.2310	73.0	0.01014
4			0.2305	72.8	0.01011
5			0.2300	72.6	0.01008

% RSD = 0.64 %

Excitation wavelength- 345 nm and Emission wavelength- 445 nm

Table no 13 Data for assay of nifedipine at shorter wavelength

S. No	Wt of std (g)	FI Quenched	Wt of tablet powder (g)	FI Quenched	Content of nifedipine (g)
1	0.025	71	0.2301	72.4	0.01048
2			0.2309	71.0	0.01028
3			0.2300	72.0	0.01042
4			0.2308	71.0	0.01028
5			0.2306	71.6	0.01036

% RSD= 0.85%

For Excitation wavelength- 355 nm and Emission wavelength- 455 nm

Table no 14 Data for assay of nifedipine at longer wavelength

S. No	Wt of std (g)	FI Quenched	Wt of tablet powder (g)	FI Quenched	Content of nifedipine (g)
1	0.025	70.2	0.2300	70.0	0.01025
2			0.2306	69.8	0.01022
3			0.2305	70.0	0.01025
4			0.2302	70.1	0.01026
5			0.2300	70.3	0.01029

% RSD= 0.24 %

RUGGEDNESS :

Ruggedness can be defined as the intrinsic resistance to the influences excerted by operational variable. The ruggedness of the method is the degree of precision of test results obtained by analysis of the samples under a variety of normal test conditions, such as different analysts, instruments and columns.

Change of analyst :

The same procedure for assay of Nifedipine tablet was repeated by another analyst.

Dilution chart for standard solution :

25mg pure Nifedipine * 25ml (1000 mg / ml)

2.5ml * 25ml (100 mg / ml)

→ *mg / ml*

3ml * 25ml (12)

+

3 ml Quinine Sulphate

Dilution chart for tablet powder :

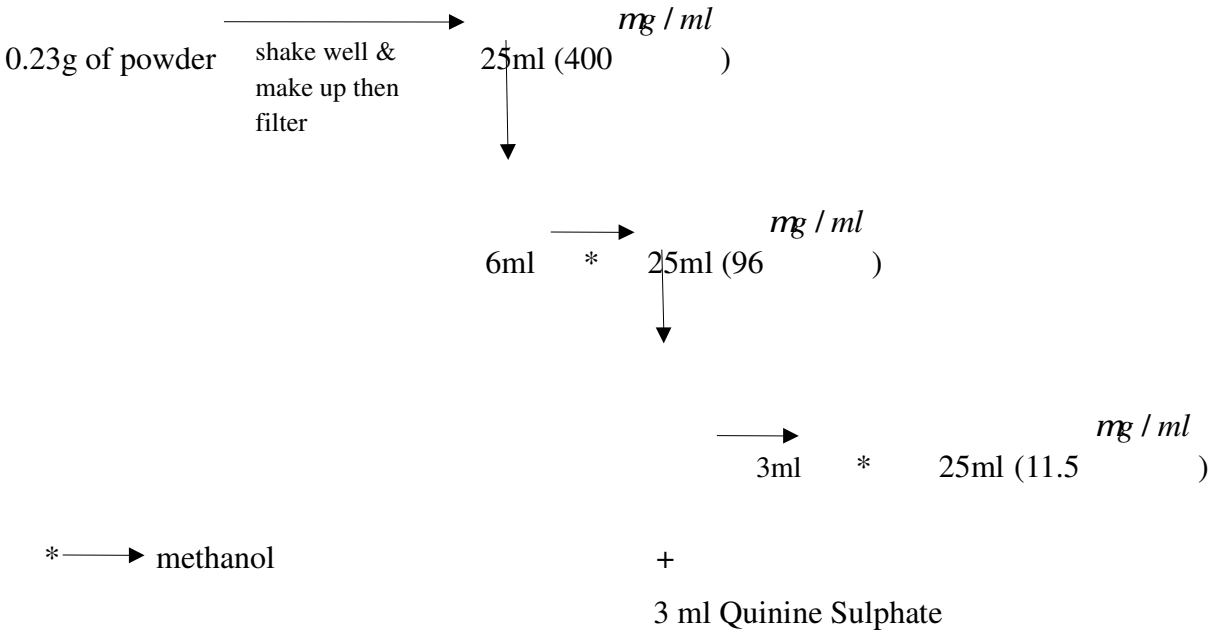


Table no 15 Data for assay of tablet by analyst I

S. No	Wt of std (g)	FI Quenched	Wt of tablet powder (g)	FI Quenched	Content of nifedipine (g)
1	0.025	74	0.2303	73.2	0.01016
2			0.2302	73.4	0.01019
3			0.2310	73.0	0.01014
4			0.2305	72.8	0.01011
5			0.2300	72.6	0.01008

% RSD = 0.64%

Table no 16 Data for assay of tablet by analyst II

S. No	Wt of std (g)	FI Quenched	Wt of tablet powder (g)	FI Quenched	Content of nifedipine (g)
1	0.025	76.5	0.2305	76.0	0.01021
2			0.2306	76.4	0.01026
3			0.2309	75.6	0.01015
4			0.2303	76.5	0.01028
5			0.2300	76.2	0.01024

% RSD = 0.46%

Precision :

Precision is the measure of the degree of repeatability of analytical method under normal operation and is normally expressed as % RSD for the statistically significant number of samples.

Three terms are widely used to describe the precision of a set of replicate data including SD, Coefficient of variation and standard error of mean.

Standard deviation (SD)= $\sqrt{\frac{\sum (x - \bar{x})}{n-1}}$

Where ,

x – Observed value

$\bar{x}$ - Arithmetic mean

n – Number of observations

The square of standard deviation is called variance. The precision is assessed by co-efficient of variation which is related to standard deviation.

Coefficient of variation (% RSD) = $\frac{SD}{\bar{x}}$ x100

Where ,

SD – standard deviation

$\bar{x}$ – Arithmetic mean

Standard Error of mean (SEM) = $\frac{SD}{\sqrt{n}}$

Where ,

SD – standard deviation

n – Square root of number of observation

Table no 17 Data for precision study

S. No	Brand name	Standard Deviation	Coefficient of variation (%RSD)	Standard Error of Mean (SEM)
1	**Nifedine**	**0.03826**	**0.42**	**0.01913**

UV Spectrophotometric Determination of Nifedipine Using Methanol

Instruments Used :

UV- visible double beam Spectrophotometer (Perkin Elmer EZ 301)

Analytical electronic weighing balance (Shimadzu)

Chemicals Used :

Methanol - S.D. Fine Chemicals, Mumbai

Tablet Formulation :

Nifedine Tablets -10 mg

METHOD DEVELOPMENT

Establishment of various parameters for the drug nifedipine :

Selection of λ-max :

Preparation of Standard Stock Solution :

Standard stock solution was prepared by weighing 20 mg of nifedipine pure drug and transferring into a 50ml flask. The drug was dissolved in small volume of methanol and the volume was made upto 50 ml with methanol to get a concentration of 400 mg/ml.

Preparation of Standard Solution :

From the standard stock solution, pipetted out 2 ml solution, into a 25 ml standard volumetric flask, and the volume was made upto 25 ml with methanol to get a concentration of 32 mg/ml .

Dilution Chart :

0.02 gm of pure Nifedipine * 50 ml (400 mg/ml)

* Methanol 2 ml * 25 ml (32 mg/ml)

Selection of Wavelength:

The standard solution of nifedipine was scanned in the region of 200 - 400 nm to get absorption spectrum of the compound. The drug exhibited a maximum absorbance at 330 nm in methanol and hence this wavelength was selected for further studies. The absorbance data were presented in table no 1

Table no 18 Data for the absorption Spectrum of Nifedipine in Methanol

S. No	Wavelength	Absorbance
1	200	1.824
2	210	1.369
3	220	1.136

4	230	1.361
5	240	1.423
6	250	0.911
7	260	0.502
8	270	0.359
9	280	0.317
10	290	0.298
11	300	0.309
12	310	0.363
13	320	0.406
14	330*	0.418
15	340	0.410
16	350	0.388
17	360	0.354
18	370	0.313
19	380	0.264
20	390	0.204
21	400	0.138

Graph No.4 Absorption spectrum for Nifedipine in Methanol

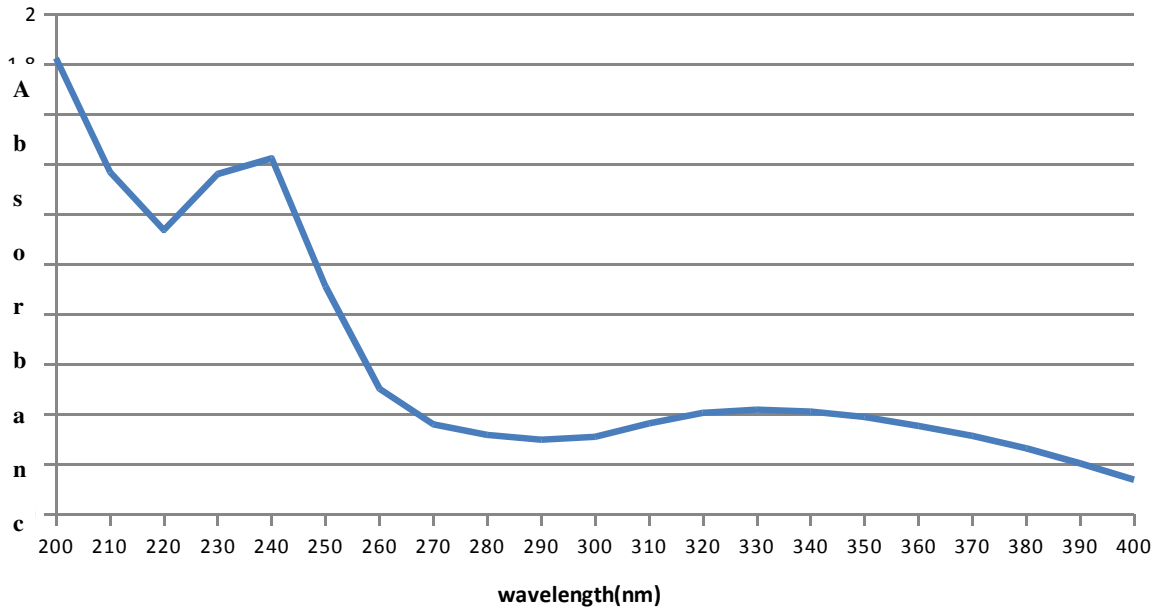


Table No.19 System Parameters

WAVELENGTH SCAN	
Data Mode	**Absorbance**
Start WL (nm)	400.00
Stop WL (nm)	200.00
Scan Speed (nm/min)	100
Slit Width (nm)	1.5
Path Length (nm)	10.0

Construction Of Calibration Curve :

0.025 gm of nifedipine pure drug was accurately weighed and transferred into 25 ml standard volumetric flask. The volume was made up to 25 ml with methanol. From this 5ml was pipetted out into a 50 ml standard flask and the volume was made up to 50 ml with methanol. From this solution different aliquots ranging from 2 - 10 ml were transferred into a series of 25 ml standard volumetric flask. The volume were made up with methanol. The absorbance of the solution were measured at wavelength 330nm. The scheme of dilution for the preparation of series of standard solution was presented below and the data for calibration curve is given in table no 3

Dilution Chart Of Calibration Curve For Nifedipine :

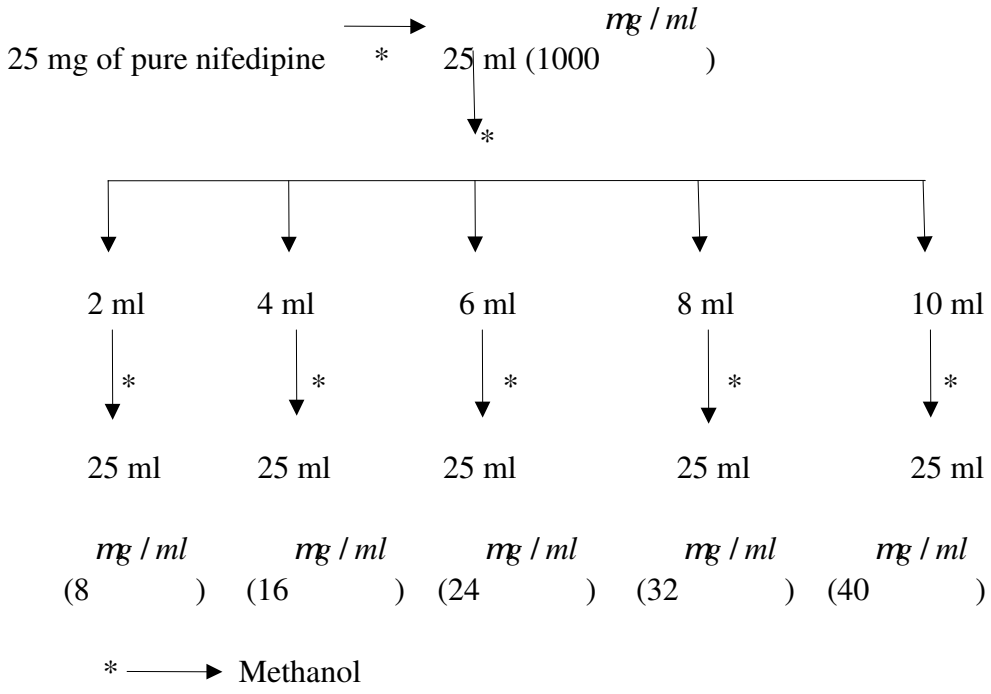
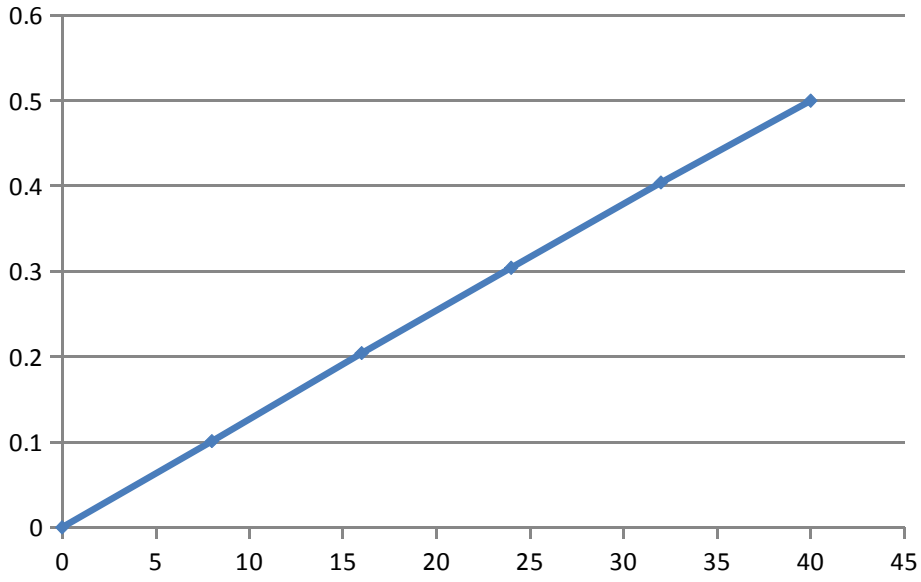


Table No.20 Data For Calibration Curve Of Nifedipine Using Methanol

S. No	Concentration (µg / ml)	Absorbance
1	8	0.101
2	16	0.204
3	24	0.304
4	32	0.404
5	40	0.500

Graph No. 5 Calibration Curve of Nifedipine Using Methanol



Correlation Coefficient = 0.999926

Limit of detection (LOD) :

The limit of detection an individual analytical procedure is the lowest amount of analyte in the sample which can detected but not necessarily quantitated as an exact value. The limit of detection (LOD) may be expressed as;

$$LOD = \sigma\ 3.3 / S$$

Where
σ = the standard deviation of the response
S = the slope of the calibration curve of the analyte

Limit of quantitation (LOQ) :

The limit of quantitation of an analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy.

Limit of quantitation (LOQ) can be expressed as;

$$LOQ = \sigma 10 / S$$

Where,
σ = standard deviation of the response
S = slope of the calibration curve of the analyte

Table No. 21 Data for LOD and LOQ

S. No	Name of the drug	LOD	LOQ
1	Nifedipine	0.0152114	0.0460952

Assay :

Preparation Of Standard Nifedipine Solution :

About 0.02 gm of pure Nifedipine powder was accurately weighed and transferred into a 50 ml standard volume flask. The drug was dissolved in a small volume of methanol and the volume was made up to the mark with methanol. From this solution, 2 ml of solution was pipetted into a 25 ml volumetric flask and the volume was made up with Methanol.

Dilution Chart for Standard Nifedipine solution :

0.02 gm of Nifedipine * 50 ml (400 mg / ml)

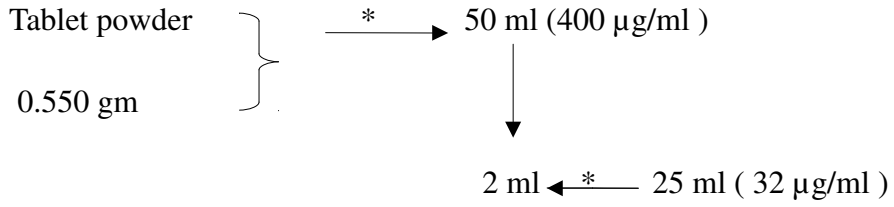
2 ml * 25 ml (32 mg / ml)

* ——► Methanol

Preparation of Test Nifedipine Solution :

Twenty tablets were weighed and the average weight of the tablet was calculated. The tablets were powdered with a help of mortar and pestle. The tablet powder equivalent to about 0.023g of the active component was accurately weighed and transferred into a 50 ml standard volumetric flask. About 30 ml of methanol was added and the flask was kept in a warm water bath for extraction of the drug. The liquid was cooled and the final volume was made up with methanol. The solution was clarified by filtration through Whatmann filter paper. 2 ml of filterate was pipetted into a 25ml standardflask and diluted with methanol to the mark.

Dilution Chart For Test Nifedipine Solution :



* ◄——— Methanol

Calculation :

Absorbance of the standard and test solution were measured at 330 nm against methanol as blank. The content of nifedipine in a tablet of average weight was calculated as follows

The content of Nifedipine is present in each tablet of average weight is

$$\frac{\text{Abs of test}}{\text{Abs of std}} \equiv \frac{\text{Wt of Std}}{50} \equiv \frac{2}{25} \equiv \frac{50}{\text{wt of tab powder}} \equiv \frac{25}{2} \equiv \text{Avg wt of tablet}$$

The data for the assay of nifedipine tablet is given in tablet no 5

Table No. 22 Data for Quantitative Determination of Nifedipine tablet

Label claim - 10 mg

S. No	Wt of Std (gm)	Abs of std	Wt of Test (gm)	Abs of test	Avg Wt (gm)	Content of tab (gm)	% Label claim
1	0.0203	0.414	0.5500	0.489	0.22753	0.00991	99.19
2			0.5501	0.497		0.01007	100.7
3			0.5500	0.496		0.01006	100.6
4			0.5502	0.489		0.00991	99.15
5			0.5500	0.496		0.01006	100.6

% RSD = 0.84 %

Excipient Studies :

Excipient studies was done with the excipient that are normally encountered in tablet formulation. The excipients were selected as talc, lactose and magnesium stearate. The excipient was accurately weighed and extracted with in methanol for 10 minutes and the procedure was followed as per the tablet assay. The absorbance of the solution was measured at a wavelength of 330 nm using methanol as a blank.

Table No. 23 Data for Interference Studies

S. No	Name of excipient	Wt of excipient (g)	Absorbance
1	Talc	0.5500	0.000
2	Lactose		
3	Magnesium stearate		

Recovery Studies :

Procedure :

Recovery studies are performed to know experimental procedure is free from interference. The excipient mixtures used such as Lactose (5 gm), Talc (500 mg) and magnesium Stearate (100 mg).

To a accurately weighed amount of excipient a known amount of pure drug nifedipine was added into a 50ml standard volumetric flask, then extracted with methanol and dilution was made up to 50ml and filtered. 2ml of solution was pipetted into a 25ml standard flask and the volume was made up with methanol. The same procedure was repeated by

changing the amount of pure drug. The recovery studies were performed at 3 different amounts of nifedipine spiked such as 20mg, 25mg and 30mg.

The standard solution was prepared by omitting the excipient mixture. The dilution charts for 3 different spiking level and standard are shown as follows,

Dilution Chart For Recovery Studies For Nifedipine tablet :

a) Dilution For Standard :

0.02 gm of pure drug ← * — 50 ml

↓

2 ml ← * — 25 ml

* ← Methanol

b) Dilution for I spiking level (20mg) :

0.02 gm of pure drug + 0.2 gm of excipient mixture ← * — 50 ml

↓

2ml ← * — 25 ml

c) Dilution for II spiking level (25 mg):

0.025 gm of pure drug + 0.2 gm of excipient mixture ← * — 50 ml

↓

2ml ← * — 25 ml

d) Dilution for III spiking level (30 mg) :

0.03 gm of pure drug + 0.2 gm of excipient mixture ← * — 50 ml

↓

2ml ← * — 25 ml

Table No. 24 Recovery Studies at I spiking level :

S. No	Wt of std (gm)	Abs of std	Wt of drug spiked to excipient (g)	Absorbance of test	% Recovery
1	0.0213	0.414	0.0212	0.416	100.95
			0.0216	0.418	99.56
			0.0218	0.419	98.88

Table No. 25 Recovery Studies at II spiking level :

S. No	Wt of std (gm)	Abs of std	Wt of drug spiked to excipient (g)	Absorbance of test	%Recovery
1	0.0219	0.414	0.0258	0.484	99.2
			0.0252	0.488	102.4
			0.0253	0.497	101.5

Table No. 26 Recovery Studies at III spiking level :

S. No	Wt of std (gm)	Abs of std	Wt of drug spiked to excipient (g)	Absorbance of test	%Recovery
1	0.0218	0.408	0.0304	0.576	101.2
			0.0306	0.574	100.2
			0.0306	0.574	100.2

ROBUSTNESS :

The Robustness is a measure of method capacity to remain unaffected by small, deliberate variations in method parameters and provides an indication of method reliability during normal use.

Change of Analytical wavelength:

The procedure for assay of nifedipine tablet was repeated by changing the analytical wavelength ± 3nm. The wavelength used to measure the content of nifedipine tablet was 327 nm, 330 nm and 333 nm respectively.

Table No. 27 Data for assay of Nifedipine tablet at 330 nm as wavelength :

S. No	Wt of Std (gm)	Abs of std	Wt of Test (gm)	Abs of test	Content of tab (gm)	% Label claim
1	0.0203	0.414	0.5500	0.489	0.00991	99.19
2			0.5501	0.497	0.01007	100.7
3			0.5500	0.496	0.01006	100.6
4			0.5502	0.489	0.00991	99.15
5			0.5500	0.496	0.01006	100.6

% RSD = 0.84 %

Table No. 28 Data for assay of Nifedipine tablet at 327 nm as wavelength :

S. No	Weight of Std (gm)	Absorbance of Std	Weight of tablet powder (gm)	Absorbance of test	Content of tablet (gm)	%Label Claim
1	0.0200	0.404	0.5500	0.485	0.01008	100.8
2			0.5503	0.486	0.01012	101.2
3			0.5500	0.481	0.01000	100
4			0.5502	0.495	0.01000	100
5			0.5500	0.476	0.00992	99.22

% RSD = 0.77%

Table No. 29 Data for assay of Nifedipine tablet at 333 nm as wavelength :

S. No	Weight of Std (gm)	Absorbance of Std	Weight of tablet powder	Absorbance of test	Content of tablet (gm)	%Label Claim

			(gm)			
1	0.0200	0.404	0.5500	0.484	0.01008	100.8
2			0.5503	0.481	0.01002	100.2
3			0.5500	0.484	0.01008	100.8
4			0.5502	0.485	0.0101	101
5			0.5500	0.476	0.009922	99.22

% RSD = 0.72%

RUGGEDNESS :

Ruggedness can be defined as the intrinsic resistance to the influences excerted by operational variable. The ruggedness of the method is the degree of precision of test results obtained by analysis of the samples under a variety of normal test conditions, such as different analysts, instruments and columns.

Change of Analyst :

The procedure for assay of nifedipine tablet was repeated by another analyst to determine the ruggedness of nifedipine tablet.

Table No. 30 Data for assay of Nifedipine tablet by another analyst :

Analyst I :

S. No	Wt of Std (gm)	Abs of std	Wt of Test (gm)	Abs of test	Avg Wt (gm)	Content of tab (gm)	% Label claim
1	0.0203	0.414	0.5500	0.489	0.22753	0.00991	99.19
2			0.5501	0.497		0.01007	100.7
3			0.5500	0.496		0.01006	100.6
4			0.5502	0.489		0.00991	99.15
5			0.5500	0.496		0.01006	100.6

% RSD = 0.84%

Analyst II :

S. No	Wt of Std (gm)	Abs of std	Wt of Test (gm)	Abs of test	Avg Wt (gm)	Content of tab (gm)	% Label claim
1	0.0202	0.410	0.5502	0.497	0.22753	0.010126	101.26
2			0.5500	0.499		0.010170	101.7
3			0.5504	0.497		0.010122	101.2
4			0.5501	0.491		0.010005	100.05
5			0.5500	0.496		0.010109	101.09

% RSD = 0.60%

Precision :

Precision is the measure of the degree of repeatability of analytical method under normal operation and is normally expressed as % RSD for the statistically significant number of samples.

Three terms are widely used to describe the precision of a set of replicate data including SD, Coefficient of variation and standard error of mean.

Standard deviation (SD)= $\sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$

Where ,

x – Observed value

$\bar{x}$ - Arithmetic mean

n – Number of observations

The square of standard deviation is called variance. The precision is assessed by co-efficient of variation which is related to standard deviation.

Coefficient of variation (% RSD) = $\frac{SD}{\bar{x}} x\,100$

Where ,

SD – standard deviation

X – Arithmetic mean

Standard Error of mean (SEM) = $\frac{SD}{\sqrt{n}}$

Where ,

SD – standard deviation

n – Square root of number of observation

Table No. 31 Data for precision study

S. No	Brand name	Standard Deviation	Coefficient of variation (%RSD)	Standard Error of Mean (SEM)
1	**Nifedine**	**0.000084083**	**0.8406**	**0.0000376**

RESULTS AND DISCUSSION

Quencho fluorimetric determination of nifedipine using quinine sulphate as a Probe moiety

Nifedipine is chemically dimethyl 1,4-dihydro 2-6-dimethyl 1-4-(2-nitrophenyl) pyridine-3,5 dicarboxylate. It is used as antianginal and antihypertensive. The molecule of nifedipine contains nitro group which is an electron withdrawing group. Therefore it can quench the fluorescence of quinine sulphate. The attempts were made to develop quenchofluorimetric method with quinine sulphate as probe moiety.

Quinine sulphate exhibits the fluorescence in 0.05M sulphuric acid and also in methanol. Its fluorescence intensity was found to be linear in the concentration range of 0.1 – 0.5 µg/ml. Quinine sulphate has excitation maxima at 350 nm and emission maxima at 450 nm. The excitation and emission wavelength were found from its activation and emission spectrum.

The drug was mixed with the selected concentration of quinine sulphate to measure the quenching. The concentration of quinine sulphate needed for the quenching study was fixed at 0.48 µg/ml from the calibration data. To obtain the linearity in quenching, the concentration of nifedipine was selected in the concentration range of 4-20 µg/ml. The mixture containing quinine sulphate with the concentration of 0.48 µg/ml with nifedipine conc ranging from 4-20 µg/ml were excited at 350 nm and residual filuorescence was measured at 450 nm. The quenching is found to be linear for nifedipine in the range of 4-20 µg/ml.

The assay of the nifedipine tablet was carried out. The average content of tablet was 0.010136g.

The percentage recovery was found to be 98.2 % - 100.0 % from recovery studies. Hence this method was free from interference and accurate. Accuracy of the assay method is also known by comparing the results of the proposed method with the assay result obtained from UV spectrophotometric method. The % RSD for the fluorimetric assay 0.42 %.

The % RSD for UV spectrophotometric assay 0.8406 %.

The method is robust which is known by carrying out the assay of tablet at different excitation and emission wavelength slightly different from that of analytical wavelengths.

The % RSD for assay of tablet at shorter wavelengths 0.85% and at longer wavelengths 0.24%.

The method has ruggedness which was determined by analysing the tablet formulation by second analyst. The % RSD for analyst I was 0.42% and the % RSD for analyst II was found to be 0.46% respectively.

The % RSD for all determinations assay of tablets, robustness, and ruggedness are within the prescribed limits.

The LOD for the drug from calibration curve was found to be 0.03083 and LOQ was found to be 0.093449.

Precision was computed from the assay value. % RSD was calculated and the value was 0.42% for assay of tablet. The data for precision study was given in table 17.

The result of interference studies showed that the excipients present in the tablet formulations have no effect in the quenching of fluorescence of probe moiety.

The optimum condition for the fluorescence studies were also carried out to set the concentration of quinine, desirable solvent, pH and temperature condition.

Hence this developed method can be used for routine analysis of nifedipine bulk powder and its tablet formulation.

UV spectrophotometric Determination of nifedipine using Methanol

Under the experimental conditions expressed, calibration curve, assay of tablets, recovery studies, change of wavelength, different analyst were performed. The nifedipine was used as antihypertensive drug. Methanol was used as solvent. Under the experimental conditions the absorbance of nifedipine exhibited λ-max at 240 nm and 330 nm. The longer wavelength has been selected as the interference will be minimum at longer wavelength. The calibration curve shows that Beer's law was obeyed in concentration range of 8-40 µg/ml. The assay of nifedipine was carried out at analytical wavelength of 330 nm. The average content of nifedipine tablet was 0.00984g. Recovery studies were carried out at three different spiking levels 100%, 125%, and 150% and % recovery ranged from 99.22-101.5%. The LOD and LOQ was found from the

calibration curve of nifedipine. LOD was found to be 0.0152114 and LOQ was found to be 0.0460952.

The precision was computed from the tablet assay and the % RSD was 0.84% and the values are within the limit. The excipients did not exhibit any interference in assay of tablet showing specificity of the method. The data for precision study was given in table 31.

The method is robust which is known by carrying out the assay of tablet at different wavelengths slightly different from that of analytical wavelengths.

The % RSD for assay of tablet at shorter wavelengths 0.77% and at longer wavelengths 0.72% respectively.

The method has ruggedness which was determined by analysing the tablet formulation by second analyst. The % RSD for analyst I was 0.84% and the % RSD for analyst II was found to be 0.60% respectively.

The % RSD for all determinations assay of tablets, robustness, and ruggedness are within the prescribed limits.

Hence this developed method can be used for routine analysis of nifedipine tablet.

CONCLUSION

From the data obtained from the above result we concluded that the methods were very simple and accurate. When compared to separation techniques such as chromatography the developed methods were simple cost effective and adequately sensitive. Hence the proposed methods were found to be convenient and suitable for adopting analysis of nifedipine in bulk drug and its formulation.

Nifedipine was selected for the quenchofluorimetric determination. It is an indirect spectrofluorimetric method. So different fluorescent probe moieties could be adopted for the assay of the drug that has the quenching ability. Quinine sulphate was found to be suitable probe moiety. The experimental conditions are as follows,

Excitation wavelength and emission wavelength were selected.

Determination of linearity range for the drug.

Checking the interference due to excipients in the assay procedure.

The proposed assay procedure is validated and the accuracy of the method was established from the recovery studies and also by comparing the results with the alternate analytical method being UV-Visible spectrophotometric method.

Precision of the method was tested by the reproducibility of the results and computition of % RSD which is low.

Fluorescence method is being a sensitive analytical technique which has been adopted in present work.

This method could be used in establishing the quality control of any formulation that contain nifedipine.

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